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MENDELIAN POPULATIONS AND THEIR EVOLUTION[1]

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THE best view of a mountain range is usually obtained from a distance; at close quarters minor peaks, and even hills, obstruct the vista of the whole range. To understand man and his universe is the goal of the scientific movement; this goal must always be kept in view, in order that we do not mistake means for the end. Well-authenticated facts are the life blood of science, and gathering them will always remain the principal function of scientific research. But science is more than a mass of facts; it is a meaningful system of significant facts. Facts taken out of their context, showy methods to solve meaningless problems, and learned terminologies for conceptual trivialities are often amusing to play with, but they stultify the work of a scientist. Integration of the results obtained by individual scientists and by various disciplines is therefore an important function which should be performed. The stones should be fitted to form an intelligible mosaic. The general view of the world unfolded by science should be kept before our eyes in order that scientific work be directed purposefully and effectively. Now, experience has shown that, at least in biology, generalization and integration can best be made by scientists who are also fact-gatherers, rather than by specialists in biological speculation. The chief aim of

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the American Society of Naturalists is to promote this generalization and integration.

Because it ties together the greatest number of most diverse facts into a simple and meaningful system, the theory of organic evolution is by far the most significant generalization which has yet emerged from the biological sciences. In the light of this theory, living beings are no longer considered as products of fortuitous accidents, or the caprice of some deity. The living world as we see it today is the outcome of a long historical development enacted during approximately one billion years of earth's existence, and still continuing to occur before our eyes.

Furthermore, the causes of this evolutionary development happen to appeal to our reasoning faculties. They seem to us to make sense. Darwin was the first to suppose, and his view has been borne out by the subsequent developments of the evolutionary thought, that organisms exhibit evolutionary changes because they become adapted, through natural selection, to live in different environments. The existence of environments unoccupied by life, or of inefficiently exploited ecological niches, constitutes a challenge to which the protoplasm may respond by evolutionary inventions of new kinds of organisms adapted to the demands of these environments. The alga *Sphaerella nivalis,* which lives on patches of permanent snow in high mountains, or *Phormidium bijahense* and *Oscillaria filiformis,* which occur in the hot springs of Yellowstone at 85° C. (Copeland, 1936), are among the more spectacular responses to apparently impossibly difficult challenges. But organic diversity as a whole must also be regarded as an answer of living matter to the diversity of environments which exist in the world.

Darwin's theory of natural selection has pointed the way to explanation of adaptive evolutionary changes. But a causal analysis of such changes became possible only in our day, because of the great discovery made by Mendel some 85 years ago, to the rediscovery of Mendel's work 50 years ago, and to the subsequent development of Mendel's prin-

ciples. This causal analysis is still far from complete; even so, it represents one of the signal achievements of biology during the first half of the twentieth century.

Darwin pointed out that differential survival of the better-adapted variants, and elimination of the less well-adapted ones, should lead to a gradual rise of the level of adaptedness of species in which such differential reproduction takes place. But Darwin realized that the process which he postulated can occur only in species which possess a supply of heritable variations in fitness to survive in existing environments. He concluded, quite correctly as we know, that most existing species do possess such a supply. But he realized that the origins of this supply had to be elucidated by further work. Mendel's discovery supplied the key to such elucidation. Hardy and Weinberg in 1908 used it to open the first lock. Investigation of the mutation process in Drosophila by Morgan and his followers created a solid base for further advance. The next step was taken by Chetverikov in 1926. His paper was published only in Russian, and it remained unknown to Fisher (1930) and to Wright (1931), who, together with Chetverikov, may be considered founders of the modern analysis of evolutionary phenomena.

The pre-Mendelian view, which was of necessity accepted by Darwin, was that the heredity of the offspring is a blend, a fusion product, of the heredities of the parents. If this were correct, sexual reproduction would devour and consume the heritable variation present in panmictic populations at a prodigious rate, namely, it would reduce the genotypic variance by one-half in every generation. At this pace of expenditure, a population would soon come to consist of genotypically uniform individuals. It would become a pure race, in which selection would cease to operate, unless, of course, genotypic variance were to arise anew at a rate at least comparable to its destruction.

It is perhaps fortunate that Darwin did not make the calculations which would have revealed to him this most serious of objections against his theory. For the difficulty

is now known to be spurious, because of Mendel's demonstration that the heredity transmitted from parents to offspring is an aggregate of genes, which do not blend but which segregate. The importance of this fact for evolution is immense. Sexual reproduction does not erode and level off, but on the contrary conserves, hereditary variability. Every sexual species accordingly possesses a gene pool, in which each gene may be represented by a certain number of alleles, and each chromosome by one or more structural variants. The frequencies of each allele and each chromosome variant in the gene pool remain constant from generation to generation, unless mutation, selection, or genetic drift intervene to alter them.

The classic way to study a species, or a part of it, is to determine the modes or averages for as many traits as possible, in as large a sample of its representatives as practicable. The resulting system of averages is taken to be a common property of the species as a whole, and is believed, at least by implication, to characterize the ideal species type. This conception of species type was logical so long as the heredity of the offspring was supposed to be an alloy of the heredities of the parents. The species type would then have been the limiting condition towards which the species would gravitate owing to sexual reproduction. But with gene heredity, a system of character averages has no real meaning, in the sense that it is not a property of any spatio-temporal object. Such a system of averages is a statistical fiction which, to be sure, may be very useful for purposes of description and cataloguing. Building a convenient catalogue of organisms is one of the tasks of biological systematics. On the other hand, a biologically realistic description of a species or a race should, theoretically, indicate the frequencies in its gene pool of gene alleles and chromosomal variants. At the present state of knowledge, such descriptions are admittedly too difficult to make to be useful in practical systematics. It must, however, be established as a principle that, in living things, diversity and variability are more fundamental than types and averages.

It is a habit of thought fostered by the exigencies of catalogue making, to regard individuals of a species as more or less perfect incarnations of a species ideal. This habit stems ultimately from Platonic philosophy and from scholastic theology. It conflicts with Mendel's findings, is basically anti-evolutionist, and is responsible for much confusion in biological thinking.

Species and races of practical systematics are categories of man-made classification. But it happens to be convenient to delimit species and races in such a way that they usually coincide with certain spatio-temporal entities, which are integrated systems of genotypes bound together by having access to common gene pools. It is important, then, to distinguish between systematic categories, set up for practical purposes of catalogue making, and the underlying spatio-temporal entities. These latter may be referred to as Mendelian populations. *A Mendelian population is a reproductive community of sexual and cross-fertilizing individuals which share in a common gene pool.*

The apprehension of Mendelian populations is made difficult by the compound nature of many of them. The biological species is the largest and most inclusive Mendelian population. Supraspecific groupings, such as subgenera, genera, etc., do not possess common gene pools, and consequently do not have the biological reality of Mendelian populations. But species are differentiated into complexes of subordinate Mendelian populations, which may be referred to as subspecies, races, or local populations. Each of these subordinate gene pools may, like the gene pool of the species, be uniquely characterized in terms of frequencies of gene alleles and chromosome variants. The smallest Mendelian populations are panmictic units (Wright, 1943), which are groups of individuals any two of which have equal probability of mating and producing offspring (provided, of course, that they are of opposite sex and are sexually mature). Panmictic units are integrated into more or less complex systems of Mendelian populations, which culminate in species.

Mendelian populations can be recognized as separate entities even if they are quite similar genetically. Genetically similar Mendelian populations may have separate gene pools because these populations are isolated from one another on different islands or by other means. By contrast, a systematist recognizes the existence of two or more taxonomic groups only if he finds genetic difference between them. The first question asked about taxonomic groups is: What traits are common to individuals within a group but differ in individuals of different groups? The first question asked about a Mendelian population is: What is its breeding structure?

The distinction between Mendelian populations and taxonomic groups can be illustrated even more vividly by considering the situation in organisms which reproduce asexually, by parthenogenesis, or by self-fertilization. If these methods of reproduction are not facultative (as they often are), but are established to the exclusion of cross-fertilization, the organisms concerned can not form Mendelian populations in the above-defined sense. For example, a clone of bacteria in which no sexual fusions occur is not a reproductive community and has no common gene pool. Individuals of a clone are genotypically alike, barring mutation; yet, a clone has no biological unity, except retrospectively by virtue of common descent from a single individual. By contrast, members of a Mendelian population have a continuous biological bond, because of the occurrence of matings in the reproductive community. Considered biologically, such a species as *Homo sapiens* or species of Drosophila flies or of birds are quite different phenomena from the clusters of clones which are referred to as "species" in bacteria, fungi imperfecti, or in obligatory apogamic plants. Nevertheless, systematists find it convenient to use the same taxonomic categories and the same descriptive techniques for all organisms.

By far the most complex system of Mendelian populations exists in the human species. Because of this complexity, anthropologists and geneticists are only beginning

to learn how to disentangle and study these populations, or isolates, as they are often referred to in man. Like many other biological species, man is geographically polytypic, *i.e.*, composed of major and minor geographic populations, or races, which differ in frequencies of many genes. But in addition to the geographic races, man has evolved national, linguistic, religious, economic, and other cultural isolates. Furthermore, human isolates do not form hierarchies of inclusive and subordinate isolates, as animal and plant populations usually do. For example, economic isolates may cut across linguistic boundaries, and religious isolates may overlap geographic ones. The lack of a clear idea of what constitutes a Mendelian population, or a race, has caused a great deal of confusion in anthropology. For example, people with blue eyes, or with round or with oblong heads, or with heads shaped like some prehistoric skull, or fat people, or people convicted for crime, or sufferers from cancer or other diseases do not form Mendelian populations. It is meaningless to call such collections of individuals races, as they have sometimes been called.

Another difficulty in the study of Mendelian populations arises from the fact that they are not fixed and static, but are dynamic entities which undergo evolutionary changes. A Mendelian population may split in the course of time into two or more derived ones; conversely, once distinct populations may fuse into one. Excellent examples of genesis, divergence, convergence, and fusion of Mendelian populations can be observed in the human species. Thus, the fluidity of social isolates is quite apparent, and development of culture has led to gene exchange between geographic races. Considered biologically, the rise of tribes, nations, and castes leads to the appearance of new gene pools. Wars, migrations, and social upheavals lead usually to the breakdown of barriers between existing gene pools. A fascinating and yet almost untouched problem is to consider and to evaluate social forces from the standpoint of their evolutionary significance.

Now, the differentiation and fusion of Mendelian popu-

lations takes place gradually and continuously. Only in exceptional cases, such as the formation of polyploids, can a new reproductively isolated population become established within a generation. Situations are, therefore, quite common in all sorts of organisms in which the boundaries between populations are not sharp or are barely indicated. This unavoidably leads to a certain degree of arbitrariness in the delimitation of populations, and of races and species to which they correspond. Hence, the disagreements among authorities as to how many races a certain species consists of, or whether certain populations should or should not be considered distinct species.

The operational difficulties encountered in the delimitation of races, species, and other Mendelian populations are, thus, an inevitable result of the continuity of the evolutionary process. These difficulties have, however, led many biologists to the view that the only objective units in biology are individuals, while all supraindividual complexes are descriptive devices created by the investigator for his own ends. The antithesis to this attitude, which may, I think, be properly labelled defeatist, is the contention that complexes of individuals often reach levels of integration so advanced that they become "supraorganisms." The idea of supraorganism has been very ably developed, for example, in the recent book of Allee, Emerson, Park, Park, and Schmidt (1949). These authors are inclined to regard not only colonies of social insects and various intraspecific aggregations, but also biotic communities consisting of many species, as supraorganisms.

The validity and usefulness of the concept of supraorganism will probably depend on the implications which the term is made to carry. But it must be admitted that one of the striking facts disclosed by modern ecology is that individuals are rarely, if ever, independent of other living individuals; they are nearly always members of more or less highly integrated systems. From the evolutionary standpoint, the individual can not be considered apart from the Mendelian population of which he is a member. Men-

delian populations are the most fundamental of the integrational forms which such systems take among sexually reproducing organisms. The integrative agent is in this instance the process of reproduction itself. The fundamental nature of this cohesive force needs no emphasis, although it is a matter of opinion whether the reproductive bond in a Mendelian population can profitably be likened to the organismic integration of cells and tissues in an individual. However that may be, Mendelian populations are spatio-temporal entities. It is interesting that Mendel's discovery implicitly reaffirmed the reality of both individuals and populations in sexually reproducing organisms. An individual is not a reflection of an ideal species type, because every individual possesses a unique genotype; in sexual species with many unfixed genes the probability of two or more individuals having by chance the same combination of genes is remote. On the other hand, the sexual unions and the gene segregations which occur in every generation condition both the continuity and the changeability of the gene pool of a Mendelian population.

It is less evident, and therefore worth greater emphasis, that Mendelian populations are, to an even greater extent than individuals, units of natural selection, and therefore of adaptation and of evolutionary change. Elimination of ill-adapted individuals within a population, exemplified by the destruction of deleterious mutants, is only one of the many forms of natural selection, and this particular form, as pointed out especially by Schmalhausen (1949), is largely a conservative rather than a creative agent. On the other hand, differential reproduction of populations appears to be very important in adaptive evolution. Most interesting for the present discussion are selective processes in populations with balanced polymorphism. Populations of many species of Drosophila are mixtures of chromosomal variants differing in the gene arrangement of certain chromosomes. Flies with different gene arrangements interbreed freely, so that individuals are formed having two chromosomes of a pair with similar and with different gene

arrangements (structural homozygotes and heterozygotes).

Now, the heterozygotes are, with few exceptions, adaptively superior to the corresponding homozygotes. Adaptively inferior homozygotes are nevertheless produced in natural populations in every generation. Poorly adapted genotypes are, consequently, normal components of the species. This seemingly strange situation is, however, explained very simply. If two gene alleles, or chromosomal variants, A^1 and A^2, form a heterozygote, A^1A^2, which is adaptively superior to both homozygotes, A^1A^1 and A^2A^2, natural selection will tend to establish an equilibrium state, at which both A^1 and A^2 will be present with certain definite frequencies. The crux of the matter is that the *average fitness of an individual in the population* will be greatest when A^1 and A^2 reach equilibrium frequencies. In other words, natural selection enhances the adaptedness of the Mendelian population as a whole, at the price of continuous production of some less well-adapted individuals.

The study of Drosophila populations has disclosed not only that balanced polymorphism is a very common phenomenon in that genus but that the intensity of the selective processes involved is greater than most geneticists suspected might be the case in natural populations. How widely similar phenomena are spread in organisms other than Drosophila is for the time being an open question. Crow (1948), Dobzhansky (1950), and Brieger (1950) have inferred that heterosis in maize must be due to a kind of balanced polymorphism, and it is possible that this is also the case in other sexually reproducing species which have large effective breeding populations. It would be unprofitable at present to speculate as to the extent to which recessive hereditary diseases and genotypic inferiorities that plague human populations may represent the genetic chaff unavoidable in the production of highly fit heterozygous types. But, assuredly, this possibility must be kept in mind in studies on the genetics of human populations.

The relationships between the adaptedness of individuals and of populations are evidently complex. It is just as

platitudinous to assert that the welfare of a population depends upon that of its members as it is to say that the health of the body is determined by the soundness of its parts. Life has evolved a hierarchy of integrative levels: genes, chromosomes, cells, individuals, several orders of Mendelian populations, and of biotic communities. The existence of all levels is based ultimately on some patterns of physico-chemical reactions, as yet unknown, which result in the self-reproduction of certain molecules, or molecular aggregates, called genes. Self-reproduction is accordingly the basic phenomenon of life, because its consequence is the process of natural selection, and hence of evolution. In turn, natural selection increases the efficiency of self-reproduction and develops a diversity of self-reproducing entities capable of functioning in a variety of environments.

The structure of genes and of all products of their integration is an outcome of a long evolutionary development controlled by natural selection. Moreover, and this is fundamental, the different methods of integration are themselves a result of adaptive evolution. This is fairly generally recognized for levels up to and including the individual; for example, the formation of multicellular organisms from colonies of only loosely associated cells is clearly an adaptive step. But the same principle applies with equal force to supraindividual levels, namely, to Mendelian populations and biotic communities.

Mendelian populations owe their existence to sexual reproduction. Darwin and Weismann realized that sex is an evolutionary adaptation, but the situation could not be understood except on the basis of Mendel's discovery. It remained for Wright, Muller, Darlington, and others to develop a cogent theory. Sexual reproduction, with its accompanying mechanisms of meiosis and contrivances that promote cross-fertilization, brings forth innumerable gene combinations which are tested for fitness by natural selection. A Mendelian population is, therefore, a laboratory for experimentation with genetic materials. From this experimentation come evolutionary inventions. To be sure,

such inventions can also be made without sex, by a lucky concatenation of mutational steps. But the probability of success is vastly increased by sexual reproduction. This is why sex has become established as the prevalent method of reproduction in higher organisms. The deterioration of sexuality which has taken place in some groups, chiefly among plants, does not contradict this view. Natural selection is not a spirit endowed with foresight but a mechanism which is basically opportunistic; it favors changes that are immediately useful, regardless of their eventual harmfulness. The advantages of an assured seed set, and other temporary benefits discussed by Stebbins (1950), account for losses of sexuality in evolution.

The integration of individuals into Mendelian populations, into sexual supraorganisms if you will, is an evolutionary adaptation. The further integration of elementary Mendelian populations into populations of higher orders, such as races and species, is likewise adaptive. It can be shown that formation of races and species has become necessary owing to the great diversity of environments found on our planet. In an absolutely homogeneous and constant environment, two or more genetically different groups of organisms could not coexist indefinitely. As pointed out by Gause, this is because one kind would in the long run prove more efficient, and would outbreed and crowd out the others. A single genotype, or at most a single Mendelian population, could exist in an absolutely homogeneous universe. In such a single population, new genotypes of superior fitness might emerge from time to time, and displace the ancestral genotypes. In principle, evolution is, therefore, compatible with the existence of a single kind of organism in the universe, instead of the immense diversity of organisms which actually prevails.

However that may be, the world in which we live is far from homogeneous. The diversity in space is most obvious: different climates and biotic conditions are encountered in different parts of the world. Adaptation to this geographic diversity of environments necessitates a corre-

sponding genetic divergence of populations that are allopatric, *i.e.*, inhabit different territories. It is this divergence which gives rise to allopatric Mendelian populations which differ in frequencies of genetic variants and which are termed races. If evolution consisted of allopatric differentiation alone, its outcome would be a single species, but one which would be more or less highly polytypic, *i.e.*, split into numerous geographic races.

A multiplicity of environments occur, however, also within the ambit of activity of a living individual, or within the distribution range of its sex cells, spores, or seeds. Different foods, different microclimates, several predators, parasites, etc., may occur within small areas. Adaptation to the variety of ecological niches which therefore exist in close proximity to each other, engenders in its turn the diverse life forms which are sympatric, *i.e.*, live within territories of the order of magnitude of the distribution means of an individual (see Mayr, 1947, for further discussion of allopatric and sympatric differentiation).

Adaptive diversity of sympatric organisms takes two forms: polymorphism within a population and speciation. Sympatric organisms must, by definition, meet each other, and, if they reproduce sexually and by cross-fertilization, they may also mate and produce offspring. In the absence of genetically conditioned barriers to crossing, sexual sympatric individuals will form, then, a single Mendelian population. Such a population would comprise a variety of genotypes each of which possesses high adaptive value in some of the different locally available environments. Such a population is called adaptively polymorphic.

Polymorphism is, in fact, observed in populations of most species. It is doubtful if any sexual species is adaptively quite monomorphic. Some species and some local populations are, however, strikingly more polymorphic than others. An attractive working hypothesis is therefore that the amount of polymorphism present in a species or a population is a function of the variety of adaptive niches which this population is able to conquer and exploit; poly-

morphic species would accordingly be adaptively more versatile, and the relatively uniform ones more specialized. This hypothesis has been found to give a satisfactory account, for example, of the different degrees of polymorphism observed in local populations and species of the *willistoni* group of Drosophila in Brazil (da Cunha, Burla and Dobzhansky, 1950; Dobzhansky, Burla and da Cunha, 1950; Dobzhansky and Pavan, 1950).

Adaptive systems based on intrapopulational polymorphism have, however, a serious limitation. Adaptive types within a Mendelian population interbreed, and gene segregation takes place in the progeny. So long as the forms crossed differ in only a single or in a few genes (or in balanced polygenic complexes, such as those guarded by inversions in chromosomes of Drosophila populations), the segregation products can fit into one or another of the adaptive niches which the whole population occupies. But, with the progress of evolution of life, adaptive systems have appeared which involve integrated systems of numerous genes. Suppose, for example, that the genotype $A^1B^1C^1D^1 \ldots$ enables its possessors to be water-dwelling, and the genotype $A^2B^2C^2D^2 \ldots$ to be terrestrial animals. The recombination products $A^1B^2C^1D^2 \ldots$, $A^2B^1C^2D^1 \ldots$, etc., may be altogether disharmonious and unable to exist either in water or on land. Using Wright's very apt metaphor, the original genotypes occupy "adaptive peaks," and the recombinations fall down into "adaptive valleys."

The well-adapted genotypes, $A^1B^1C^1D^1 \ldots$ and $A^2B^2C^2D^2 \ldots$ could maintain themselves in allopatric populations and could live in different territories separated by a geographic barrier. However, if the ecological niches to which each genotype is suited should exist in both territories, then only one of them would be occupied, leaving the other niche empty, in both territories. On the other hand, should the two populations become sympatric, their members would hybridize. The resulting gene exchange and recombination would give rise to a mass of disharmonious genotypes, which would pull down the level of adaptedness of both

populations concerned. In reality, this dilemma is avoided quite simply. The populations become sympatric and occupy all the adaptive niches for which they are suited, but only because hybridization and gene exchange between them are prevented, or made rare enough, so that the adaptedness of the populations remains high. This is exactly what is observed in nature: gene exchange between sympatric Mendelian populations is reduced or suppressed by a great variety of reproductive isolating mechanisms.

The writer has suggested, as early as 1935, that species are reproductively isolated Mendelian populations. The process of speciation must, then, be regarded as an evolutionary adaptation which permits the development of immense organic diversity, particularly the diversity of sympatric species. Between one and two million species of animals and plants have developed on the earth neither to please nor to plague biologists and collectors. This diversity of species is a device which enables life to exploit the multiform opportunities offered by the environment. Speciation is accordingly a form of integration of Mendelian populations engendered by natural selection in response to the challenge of the diversity of sympatric environments.

In view of the more than century-long, and notoriously inconclusive, debate about what species are, it is gratifying that a fairly general agreement about the matter seems at last in the offing among biologists. It is recognized, except by a few conservatives, that the attainment of reproductive isolation between genetically diverging Mendelian populations is the essence of biological speciation. Reproductive isolation between populations is, accordingly, the criterion for the recognition of the specific entities which have biological meaning. Of course, it does not follow that different species are always "intersterile." It can not be too strongly emphasized that hybrid inviability and hybrid sterility are only two of the many kinds of reproductive isolating mechanisms. One could cite numerous instances in which fertile hybrids between species are known in the laboratory or in nature, or in both. What matters is not whether

hybrids can be obtained but whether the Mendelian populations do or do not exchange genes, and if they do whether at a rate which destroys the adaptive equilibrium of the populations concerned. Seasons of sexual maturity or of flowering, attraction of different species of insects for pollen transport, being members of different biotic communities in the same geographic region, weakness or lack of sexual attraction between members of different populations, are some of the reproductive isolating mechanisms which keep species genetically apart, despite the possibility of production of fertile hybrids under some circumstances. Moreover, it is a very common situation that species are kept apart not by one but by several reproductive isolating mechanisms, none of which may be absolutely effective when taken alone, but which eliminate all gene exchange when combined. An example of this situation are the sibling species *Drosophila pseudoobscura* and *Drosophila persimilis*. Hybrids between them are rather easily produced in the laboratory, and yet no hybrids have ever been found in nature, despite the side by side occurrence of the parental species in many habitats.

Not only the presence but also the degree of reproductive isolation between species will be determined by the exigencies of adaptive evolution. Gene exchange between Mendelian populations is adaptively favorable or unfavorable, depending upon the fitness of the recombination products in relation to the environment. If some of the recombination products are valuable in some environments, it is advantageous for species populations to keep open a more or less narrow channel for gene exchange. Hence the occurrence of introgressive hybridization between some species, and its absence between others. The evolutionary importance and the prevalence of introgressive hybridization have been, in this writer's opinion, greatly exaggerated by some authors. This controversial problem can not be discussed here in detail. Nevertheless it should be pointed out that no matter how common such hybridization may prove to be, the formation of reproductive isolating barriers

between Mendelian populations must be considered one of the basic phenomena of evolution. In the last analysis, speciation is an adaptive accompaniment of sexual reproduction, just as sexual reproduction is a corrective to the relative stability of the gene.

Evolution, then, is a creative process, but not in the sense of being directed by some supernatural force, as has often been explicitly or implicitly supposed by vitalists and their modern successors, the finalists. Such a direction would still amount to an inexplicable caprice of a Creator or Director, but a caprice lasting a billion years instead of the six biblical days. Evolution is creative because it involves the formation of previously non-existent coherent entities, Mendelian populations culminating in species; because these entities enable life to spread into and to exploit new environments more and more efficiently; and because evolution, like all creative processes, involves risk of failure or miscreation, in other words, partakes of the quality which, in application to human affairs, is called freedom. This gives to organic evolution a character which can be described again only by an anthropomorphic term, namely, a character of dignity.

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TAXONOMY, LANGUAGE AND REALITY

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I. SOME recent discussions in *Evolution* (Mayr, 1949; Burma, 1949) have reopened the old controversy whether species are *objectively real.*[1] The lay biologist, browsing in the literature and hoping to come on his account to a satisfactory resolution of this puzzle, is confronted with an immense record of apparently irreconcilable but at the same time irrefutable views, each claiming a basis in solid taxonomic fact. The history of this controversy, therefore, might suggest that intelligent analysis of the relevant factors presupposes intimate acquaintance with the foundations of taxonomy and intensive first-hand experience with the methods of taxonomic inquiry, and that criticism of the authoritative opinions that have been advanced is not an activity in which mere amateurs may profitably engage.

But to a stubborn inquirer the issues of this controversy sooner or later may come to appear less profound than muddled. There is much ground for the accusation that participants in it seldom have clearly formulated their ideas about the kinds of entities that are referred to by taxonomic class-expressions, or of the kinds of attributes that sensibly may be predicated of them, or of the sorts of relations that significantly may be said to hold between them. Taxonomic writing, therefore, frequently is guilty of *ambiguity of reference;* and the failure to distinguish explicitly between entities of such diverse taxonomic and logical types as organisms, classes of organisms, and classes of classes of organisms entails the sometimes very puzzling problem of deciding just what entities are being discussed. Furthermore, the liberal and semantically uncontrolled use of

[1] Detailed accounts of the controversy are given by Mayr (1942) and in the articles edited by Huxley (1941).

expressions like "objective," "real," "natural," "exist," "arbitrary," "subjective," "abstract" and "artificial" has introduced into taxonomic theory much regrettable *ambiguity of meaning*, for these are among the most equivocal of English expressions, and in taxonomic contexts it is not always easy to see in which of several equally probable but not equally defensible senses they are being employed. Finally, there does not appear to be anywhere in taxonomic literature a clear statement of the precise conditions that *would* furnish grounds for decision whether or not species, or any other taxonomic groups, are correctly to be characterized by using one or more of these expressions. Taken all together, the circumstances under which the controversy has been conducted may seem to justify the suspicion that little progress has been made because the basic issues have never been clearly conceived.

The general tenor of the controversy suggests that taxonomists in attempting to support their various theoretical views may be confusedly fighting a battle of hazily formulated private ontological dogmas when they might with greater profit be appealing to methodological rules governing the construction of acceptable taxonomic systems. This suggestion will be developed further in the body of this essay, which is offered as a preliminary step toward a clearer, less ambiguous treatment of the problems of taxonomic methodology. But it is one thing to recognize an ambiguous situation and another to rectify it, and we shall not claim to have done more than proceed a little way on the road to clarity.

II. Whatever else taxonomists may be doing, we shall suppose it correct to say that since the appearance in 1758 of the tenth edition of Linnaeus' *Systema Naturae* they have been engaged in the business of *classifying* living and extinct organisms; that is, they are concerned with constructing a system of *classes* of organisms in accordance with some more or less explicitly formulated general principles that are designed to implement certain tacit or stated

taxonomic aims. We shall take it for granted, therefore, that species, genera, families and other taxonomic groups are classes of organisms; and that familiar expressions like "Arthropoda," "Chlamydomonas," "Diptera," "Canidae" and others are *class-names.*

Now, although there has been much discussion about the "existence" of entities named by these expressions, we can not concur in the view that this is a significant biological problem. But let us inquire, nevertheless, what it is that statements to the effect that taxonomic groups of various sorts exist are asserting; let us consider, for example, the statement:

(1) Species exist.

As it stands, (1) is ambiguous: it may mean either that *all* species exist, or only that *some* (an unspecified number) of species exist. Suppose we consider the former case first. The problem now is to see just what it is that (1) may be asserting about species, if it is taken to mean:

(2) All species exist.

We know what we mean by *other* statements of this same grammatical form. By "All fish swim," for example, we mean "For any x: if x is a fish then x can swim." If (2) is translated similarly, we arrive at:

(3) For every A: if A is a species then A exists.

But unlike our statement about fish, which clearly provides us with the factual information that they are swimming things, (3) gives us no factual information whatever. The reasons are these. The negation of (3):

(4) not (For every A: if A is a species then A exists)

is *inconsistent,* for it entails the self-contradictory statement:

(5) There exists an A such that A does not exist.

Since (5) is self-contradictory, and therefore trivially false, it follows that (4) also must be false. But if (4) is false, then *its* negation, namely (3), must be *true;* and this solely

by virtue of its linguistic form, not because it asserts any empirically verifiable fact. To say, therefore, that all species exist is to utter a trivial truth that, like "all red things are colored" and "$2 \times 2 = 4$," is certifiable on purely logical grounds; and to deny that all species exist is to be self-contradictory. Taxonomists anxious to defend the view that all species exist are also committed, therefore, to the view that all genera exist and that all families exist and indeed to the view that all unicorns exist (embarrassing though this may seem to be) for to deny it is to defend an inconsistency.

Suppose, on the other hand, that by (1) is meant only that:

(6) Some species exist.

Now we might be tempted to translate (6) to read: "There exists an A such that A is a species and A exists"; for it is certainly correct to translate "Some fish swim," which has the same grammatical form as (6), to read: "There exists an x (at least one, perhaps thousands) such that x is a fish and x swims." But we shall argue that it is not a good idea to construe (6) in this way. The *subcontrary* of "Some fish swim," *i.e.*, "Some fish do not swim" means simply that "There exists an x such that x is a fish and x does not swim"; and this, whether true or false, is at least not self-contradictory. But the contrary of "Some species exist," *i.e.*, "Some species do not exist," when construed in this manner to read: "There exists an A such that A is a species and A does not exist," *is* self-contradictory, for it entails that there exists something that does not exist. If "exists" is used in this way, therefore, the subcontraries of statements like (6) have the queer property that they are inconsistent. We shall have to find some other way of employing "exists" if we are to preserve consistency in our language.

Now we all know what is meant by statements like: "Fish do not exist." We would never misconstrue this false statement to mean: "Some fish do not exist," for this is self-contradictory, as we have seen. Nor would we con-

strue it queerly to mean: "All fish do not exist," that is, to mean: "Everything that is a fish does not exist." On the contrary, we would know it to mean mistakenly but exactly: "There is no x such that x is a fish." Similarly, we are always willing to construe:

(7) Species do not exist

to mean the same as:

(8) There is *no* A such that A is a species.

Now if we were to deny (7), and to assert that species *do* exist, then we must mean:

(9) There *is* an A such that A is a species,

and this says all that legitimately can be asserted by "Species exist." Now (9), we shall claim, is a proper translation not only of (6), but exhibits the form of all existential statements like it. But note that (9), unlike "There is an A such that A is a species and A exists," does not assert that anything has the characteristic of existing, but only that something has the characteristic of being a species.

If these considerations mean anything, then the question whether taxonomic groups of various kinds exist is not very hard to settle. For, if the statement:

(10) Species do not exist

means the same as:

(11) There exists no A such that A is a species,

then (10) is *false*, for there *are* species: *Homo sapiens* is one, *Escherichia coli* is another, and *Anopheles quadrimaculatus* is another. Furthermore, if:

(12) Families do not exist

is to mean the same as:

(13) There is no A such that A is a family,

then this, too, is clearly false; for there are taxonomic families: Canidae is one, Drosophilidae is another, and Cicadidae is another. In general, *all* statements of the form of

" F's do not exist " are false, where " F " is substituted for by the name of any of the official taxonomic categories: "Species," "Genus," "Family" and the others. This being the case, all statements of the form of:

F's exist,

i.e.:

There is an A such that A is an F,

are *true,* whenever " F " is substituted for by any taxonomic category-name. In such statements, ostensible reference to existence as a *characteristic* of taxonomic groups does not occur. Certainly, no problems worthy of many decades of heated debate are posed by assertions such as this.

But the pseudo-problem of existence is not the only one that has been generated in taxonomy by the misuse of language: we may add also the question whether taxonomic groups are " abstract " entities, or whether they are objects discoverable, like organisms, " in nature." Now if it is correct to say that taxonomic groups are classes of organisms, then the question whether taxonomic groups are abstract entities is an instance of the more general question whether *classes* are abstract entities. Let us see what may be said on this topic.

One objection to the view that classes of organisms are spatiotemporal entities " in nature " is that adherence to it robs us of one of our most useful modes of expression. Among our most common expressions, in and out of technical biology, are statements like " Jones is a member of the class of humans," which are of the form of " The object x is a member of the class A." But if the view that class-expressions are names of spatiotemporal *objects* of some sort is correct, then in uttering expressions of this form we are guilty of uttering nonsense. For it is literally meaningless to say, in any usual sense of " is a member of " (see section III of this essay), that an object is a member of another object. Objections that it is not nonsense to say this seem based upon the notion (advanced independently by two different taxonomists reading an earlier version of

this paper) that species are composed of organisms just as organisms are composed of cells: according to this argument a species is just as much a concrete, spatiotemporal thing as is an individual organism, though it is of a less integrated, more spatiotemporally scattered sort. This argument sounds fairly plausible, until one reflects that it contains an equivocation upon two common meanings of " is composed of." It is true that an organism is composed of cells; it is also true, but in a different sense, that species and other taxonomic groups are composed of organisms. The relation of a cell to the organism in which it is located is the relation of part to whole. But the organism–species relation is that of member to class; and these are entirely different sorts of relations. Let us suppose, for example, that there is a cell x which is a part of an organ y, and that y is a part of an organism z. It follows that x is part of z: part–whole is a *transitive* relation, like " less than." But given that some object x is a member of some class A and that A is a member of another class F, it does *not* follow (see section III) that x is a member of F; *i.e.,* membership is *not* a transitive relation. Therefore, " is composed of " can not be construed in the same context in the sense of " has as members " and in the sense of " has as parts." But we may go further than this.

Suppose that there is a cellular organism O. Let us mean by " C " the class of all the cells of O. On the view we are refuting, O and C are identical, *i.e.:*

(1) $$O = C.$$

Now, if the view that an individual is composed of a class of parts allows us to identify the individual and the class, then we may identify O with the class M of the *molecules* which compose O, *i.e.,* to assert:

(2) $$O = M.$$

From (1) and (2) we are now entitled to infer:

(3) $$C = M,$$

i.e., to infer that the class of cells composing O and the class of molecules composing O are identical. But this is

patently absurd, for these are *mutually exclusive* classes: no cell is a molecule and no molecule is a cell. We must admit, therefore, that:

(4) $C \neq M$,

but this contradicts (3).

In general, *classes and concrete entities can not be identified* and the part–whole relation used interchangeably with the member–class relation without engendering inconsistencies. Classes are *abstract, non-spatiotemporal* entities; and statements of the form of "x is part of y" should not be confused with statements of the form of "x is a member of A," where x and y are individual physical objects and A is a class.

Appeal to the ontological dogma that classes are spatiotemporal entities "in nature" in support of the view that certain taxonomic groups are "objectively real," while others are not, is thus seen to be ill-conceived. For all classes of objects (and classes of . . . classes of objects) are abstract, non-spatiotemporal entities. *This* problem, then, is a pseudo-taxonomic one which is to be resolved by reference to the semantic structure of language, and upon which no purely biological evidence (geographical distribution, interbreeding relations, etc.) has the slightest bearing whatsoever.

The supposition made by some taxonomists that classes of organisms *could be* spatiotemporal entities seems to have at least three sources: (a) The ambiguity of expressions like "is composed of," that confuse the part–whole relation with the member–class relation; (b) The historical accidents of everyday language in which classes metaphorically are referred to as *objects; e.g.,* "the *limits* of species are *blurred,*" "the *borderline* between species," "drawing a circle *around* species," etc.; and (c) The insidiously misleading use of class-expressions in place of individual-expressions; *e.g.,* "This species nests in oak trees" for "*The members of* this species nest in oak trees," and "This species is sexually active the year round" for "*The members of* this species are sexually active the year round," to use

two actual examples from the literature that are wholly trivial in themselves, but nevertheless are indicative of the sort of linguistic ambiguity that does, finally, lead to disaster in exact theoretical discussion.

Whatever are the biologically interesting aspects of the controversy we have been discussing, it should be apparent by now that they are not associable in any literal sense with what is assertable by statements employing the expressions "objective reality in nature" and "existence." The misuse of expressions like these, as we have claimed earlier, has led taxonomists to confuse ontological pseudo-difficulties with genuine methodological problems facing them in the building of adequate taxonomic systems. It is time now that we turn to a discussion of the latter.

III. Before we can give an account of the most general features of some methodological problems confronting taxonomists, we must arrive at a clear understanding of the structural features of the system of classes they are engaged in constructing. We may begin by recalling some elementary logical distinctions that in ordinary language are expressed in various idioms of the verb "to be."

"Polaris" and "The North Star," we know, are expressions that refer to a single individual (namely, a certain bright object that appears at night above the northern horizon); and "Polaris is the North Star" is a statement that asserts this fact. Likewise, "Cicero" and "Tully" name the same individual; or, as we commonly say, "Cicero *is* Tully." In these examples, "is" is being used as a sign of *identity;* and to distinguish this usage of it from others we shall mention shortly, we shall write:

(1) $$x = y,$$

whenever we wish to assert that x and y are the same individual, using "$=$" (read "is identical with") in place of "is"; *e.g.,* "Polaris $=$ The North Star," and "Cicero $=$ Tully."

Unlike "Cicero is Tully," the sentence "Arthropoda is the largest phylum" asserts the identity of reference not of two individual-names but of two expressions referring to a

class of individuals; it tells us that "Arthropoda" and "The largest phylum" are expressions designating the same class of joint-legged invertebrates. We may write this in the manner of (1) to read: "Arthropoda = the largest phylum." In general, we shall write:

(2) $A = B$

whenever we wish to say that "A" designates the same class as "B."

A second use of "is" is illustrated by the statement: "Reynard is a fox." "Reynard," we know, refers to a single vulpine individual, while "fox" on the other hand refers to a whole *class* of such individuals. What "Reynard is a fox" is telling us, then, is that Reynard *is a member of the class of* foxes. We may write "Reynard is a fox" to read: "Reynard ϵ Fox," where "ϵ" (read: "is a member of the class") is written instead of "is a" not for esoteric reasons, but simply to distinguish this use of "to be" from its use as an identity sign. In general, whenever we want to say that an individual x is a member of a class A we can write an expression of the form of:

(3) $x \epsilon A.$

In taxonomy, for example, there are many statements having this form: "x ϵ *Homo sapiens*," "x ϵ Arthropoda," "x ϵ Artemia" and others. "x ϵ A," therefore, corresponds to the common idiom "x *is an* A"; while "x = y" corresponds to the idiom "x *is* y."

The sign of class-membership "ϵ" may stand not only between an expression designating an individual and one designating a class of individuals—as in "x ϵ A"—but also between an expression designating a class and one designating a class of classes; as, for example, in "*Homo sapiens* ϵ Zoological Species," "Artemia ϵ Genus," and "Arthropoda ϵ Phylum." In general, we may write expressions of the form of:

(4) $A \epsilon F$

to indicate membership of a class A in another class (of classes) F.

It should be noted here that given "$x = y$" and "$y = z$," it is always correct to infer "$x = z$"; but given "$x \epsilon A$" and "$A \epsilon F$" it is *not* correct to infer "$x \epsilon F$." For example, given "Jones ϵ *Homo sapiens*" and "*Homo sapiens* ϵ Zoological Species," it is not correct to infer "Jones ϵ Zoological Species." *Homo sapiens* is a class of organisms and thus may contain the organism Jones as a member, while Zoological Species is a class of *classes* of organisms (of which *Homo sapiens* is only one), and thus does not contain as a member the organism Jones, who is not a class of organisms. Membership thus differs from identity and from part–whole (as we saw in Section II) in being a nontransitive relation.

A third use of "to be" corresponds to the idiom "A's are B's"; as in "frogs are amphibians." What this latter expression says is that everything that is a frog is also an amphibian; *i.e.*, it says that for any x, if $x \epsilon$ Frog then $x \epsilon$ Amphibia. In general, when to be a member of a class A insures membership in another class B, then we shall say that A *is included in* B. If we write "$\subset$" to mean "is included in," then where A and B are classes:

(5) "$A \subset B$" means the same as "For any x, if $x \epsilon A$ then $x \epsilon B$."

In taxonomy, for example, we have the statements: "Amphibia $\subset$ Vertebrata," "Drosophila $\subset$ Insecta," and others. Notice that it would not make sense to write "Amphibia ϵ Vertebrata" for (the class) Amphibia is not a *member* of (the class) Vertebrata; since Vertebrata is a class of organisms, and the class Amphibia is not an organism, but is itself a class of organisms. In general, given "$A \epsilon F$" it is not permissible to infer that any member of A is also a member of F.

We shall now employ the three relations designated by "$=$," "ϵ" and "$\subset$" in giving a brief account of the main structural features of the present-day system of biological classification, which for lack of a better name we shall call "L" (after Linnaeus, though his system has been considerably altered in the nearly two centuries since its proposal).

There is an important distinction to be made between what we shall call "taxonomic groups" in the Linnaean system L and what we shall call "taxonomic categories" in L. By "taxonomic group" we shall mean any class in L whose members are organisms, living or extinct; *e.g.,* Felis, *E. coli,* Reptilia, Hominidae, Echinodermata and others. By "taxonomic category" on the other hand we shall mean any class in L whose members are not organisms but are taxonomic groups; *e.g.,* Species, Genus, Family, Order, Class, Phylum, Kingdom and any subdivisions of these that may be introduced.

The relations holding between an organism, the various taxonomic groups of which it is a member, and the taxonomic categories of which the groups are members, are shown diagrammatically in Table I. If while consulting this table the reader will recall what is *meant* by statements in which "ϵ," "$=$" and "$\subset$" appear, he will note that while it makes sense to say that the membership relation holds between an organism and a taxonomic group or between a taxonomic group and a taxonomic category, it would *not* make sense to say that it holds between two organisms, two taxonomic groups, two taxonomic categories, or an organism and a taxonomic category. Similarly, the inclusion relation holds only between two taxonomic groups and never between two taxonomic categories or between an organism and the group to which it belongs. A little attention should make these facts clear; but should this not prove to be the case, the reader is invited to refer to Langer (1937) for a clear detailed elementary account of the theory of classes, and to Quine (1950) and Woodger (1937) for discussion of certain other logical matters touched upon in this essay.

We may now inquire what general criteria must be satisfied by a taxonomic group if it is to be admitted into the system L.

Simply by way of convention, let us agree to call a statement of the properties which an organism x must have in

TABLE I

Relations ordering the "Linnaean" system of classification. The organism designated by "x" in column 1 is a member of every taxonomic group named in column 3. Each taxonomic group is a member of the taxonomic category whose name appears in column 5 in the same row only. x is not a member of any of the taxonomic categories named in column 5. Turning to column 1, x is of course related to itself by identity, and is a member of the class *Organism*. In column 3, each taxonomic group named is included in (but is not a member of) every taxonomic group whose name appears above its name; and each taxonomic group named is a member of the class (of classes) *Taxonomic Group*. In column 5, each taxonomic category named is a member of the class (of classes of classes) *Taxonomic Category*. No direct ordering relation holds between the various taxonomic categories, for these are given in the system simply by enumeration (in particular, neither ϵ nor $\subset$ relates them).

	1	2	3	4	5
	Organism		Taxonomic Group		Taxonomic Category
	ϵ		ϵ		ϵ
13	x	ϵ	Animalia	ϵ	Kingdom
12	=		$\subset$		
11	x	ϵ	Arthropoda	ϵ	Phylum
10	=		$\subset$		
9	x	ϵ	Insecta	ϵ	Class
8	=		$\subset$		
7	x	ϵ	Diptera	ϵ	Order
6	=		$\subset$		
5	x	ϵ	Drosophilidae	ϵ	Family
4	=		$\subset$		
3	x	ϵ	Drosophila	ϵ	Genus
2	=		$\subset$		
1	x	ϵ	Drosophila melanogaster	ϵ	Species

order to be a member of a particular taxonomic group A, a *group-description;* for example, a list of the properties which an organism must have in order to belong to the genus Daphnia, is a group-description. But we call attention to group-descriptions mostly to distinguish them from what we shall call *category-definitions.*

A category-definition, we shall say, is any list of the properties which a taxonomic group must have in order to be a member of a taxonomic category; a definition of "Phylum," for example, is a category-definition. Let us

say, further, that a taxonomic group A *satisfies* a category-definition D when, and only when, A exhibits the properties mentioned in D. Arthropoda, for example, satisfies a certain definition of "Phylum" if, and only if, Arthropoda exhibits the properties mentioned in the definition; if so, "Arthropoda ϵ Phylum" is a true statement. But this must not be construed as meaning that just *any* definition of "Phylum" would be acceptable to taxonomists, for any such category-definition must meet certain requirements of adequacy relative to the purposes for which L is constructed.

For example, if L is being constructed simply as a *catalogue* of organisms, then the category-definitions of L must insure that the groups admitted to L meet one kind of criterion of adequacy; another kind of criterion of adequacy may have to be met by the groups admitted to L if L is being constructed to organize and exhibit our knowledge of certain specified *kinship* relations holding between organisms; and category-definitions need to be framed so that their employment shall result only in the admission to L of groups whose members exhibit these relations to each other and to the members of other admitted groups.

Let us suppose there is some set P of stated ends for which L is being constructed (in particular let us suppose that the statements of P formulate the modern aim of arriving at an *evolutionary* classification of organisms); and let us call a *requirement of taxonomic adequacy* a statement of the properties which the members of any taxonomic category must have if they are to further the ends mentioned in the statements of P. Let us suppose also that a requirement of taxonomic adequacy has been specified for the members of each category. Then we shall say that a taxonomic group A *satisfies* a requirement of taxonomic adequacy T when and only when A exhibits the properties mentioned in T. Let us then call *taxonomically adequate* any category-definition D such that every taxonomic group A satisfying D also satisfies a requirement of taxonomic adequacy for the groups that are members of the category defined by D.

Now we are in a position to state in quite explicit general terms what it is that taxonomists mean to assert when they apply to some taxonomic group A in L such terms as "natural," "objective," "real" and others: they must mean that

(6) There is a D such that D is a category-definition, and A satisfies D, and D is taxonomically adequate.

And when they call some taxonomic group A in the system L "arbitrary," "subjective," "abstract," or "artificial" they must mean to say that the methodological conditions mentioned in (6) fail to hold for A. That is to say, if we are to make any sense at all of statements employing these ambiguous terms we must construe them as tacit references to rules of taxonomic procedure; not as appeals to various footless ontologies.

But if the foregoing is to stand as an acceptable analysis of such statements, then we must be able to account in terms of it not only for the generally held taxonomic view that genera, families and other groups belonging to the "higher" categories are best characterized by such terms as "subjective," "artificial," and "arbitrary," but also for the fact that there is widespread disagreement about a similar characterization of species.

Now, this analysis easily interprets the first view, that genera and other higher groups somehow occupy a taxonomic status different from that of species. It is commonly admitted by taxonomists there are no known definitions of "Genus," "Family," "Order," and so on, that are taxonomically adequate in the "evolutionary" sense—that is to say, whose use insures that among other things genera, families and orders will be established whose members bear to each other and to the members of other such groups certain specifiable evolutionary relations. There are many genera, families, etc., now listed in the Linnaean system that are arbitrary with respect to the requirements of an "evolutionary" classification; and this is sometimes expressed in the misleading ontological mode of speech by

saying that they are not "objectively real." But it is obvious that a more defensible and adequate expression of these same facts can be made by reference to taxonomic criteria.

Our analysis also provides a ready interpretation of the species-controversy. For there *are* definitions of "Species" for which a limited taxonomic adequacy may be claimed, but most if not all of them have certain features about which disagreement can arise. For example, given a definition of "Species," it may be charged (we withhold opinion here) that it is inadequate because it is *vague* (*e.g.*, the definition: "A species is a group of morphologically similar organisms" may be said to be vague, for there are situations in which, given two organisms, it would be impossible to decide save by fiat whether or not they were sufficiently similar to belong to the same species); or that it is inadequate because it is *difficult practically to apply* (*e.g.*, the definition: "A species is a maximal group of interbreeding organisms" may sometimes be difficult to apply, since it is not always an easy matter to demonstrate that a set of populations *is* such a group); or that it is inadequate because it is *not generally applicable* (*e.g.*, the last definition is inapplicable to asexually reproducing organisms and to fossil organisms); or there may be still other reasons upon which may be based the charge that it is taxonomically inadequate.

Now whether or not any of the species-definitions now available are taxonomically adequate; and whether the species and other taxonomic groups now listed in taxonomic systems do as a matter of fact meet requirements of the sort mentioned in (6) is not our business to discuss; for these are genuine taxonomic problems upon which purely methodological considerations have little or no bearing. Indeed, probably no general statement one way or another would be correct, for taxonomic analysis in future may reveal that some taxonomic groups now listed in the Linnaean system are unsuitable while others are entirely acceptable. Such analysis profitably can be carried out only with respect to (a) specified taxonomic groups, (b) speci-

fied category-definitions, and (c) specified criteria of taxonomic adequacy; that is, under conditions in which it may be clearly recognized when an answer has been obtained, which only taxonomists are competent to specify. We *shall* claim, however, that problems of this kind (irrespective of whether we have given an adequate formulation of their general nature here) are the only taxonomically relevant issues raised by the question whether taxonomic groups, be they species or whatever, are objectively real; but to most biologists these may seem to offer a sufficiently stimulating challenge.

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MARGINALIA TO McCLINTOCK'S WORK ON MUTABLE LOCI IN MAIZE

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IN a series of reports since 1946, Barbara McClintock has presented the results of her most remarkable work on so-called mutable loci in maize. Though these reports are only of a preliminary nature, the factual data as well as the interpretations are of such importance that a discussion at this moment seems to be in order, especially in view of the fact that the special features of the plant material may deter animal geneticists from studying the impact of this work upon their own. The personal reason for proposing such a discussion is that many of the data in maize show a remarkable parallelism to older work in a completely different field, namely, the analysis of intersexuality. A discussion of this parallelism, together with that of some further theoretical aspects, might help to make some parts of the new work in maize appear less unusual and *vice versa* may help to clarify some old riddles in the light of the new facts. In addition, such a discussion may show how the trend of ideas on the nature of the genic material is consistently going in a definite direction away from the classical theory of the gene.

1

Let us first extract the essential facts from McClintock's work and free it of details. It deals with so-called mutable loci in maize, which are phenotypically expressed by mosaic patches of the mutant type. This traditional description actually does not fit all the facts satisfactorily, as will soon be seen. The first case analyzed shows the following genetic features. The mutable locus is called *Ds* (from dissociation) because the "mutation" it produces consists in a break of the chromosome at the locus which marks off the

proximal third of the short arm of the ninth chromosome. The break, called the mutation, results in the production of an acentric fragment which is subsequently lost. (In the latest paper, 1950, stickiness of the *Ds* region is made responsible.) If the other chromosome is marked with recessives located in this distal two-thirds of the arm, the phenotypes of the recessives will be visible in all cells descended from the one which "mutated" if the action of the recessive is cell specific and of a recognizable type, *i.e.*, the color gene (*c*) or the waxy gene (*wx*). The time and place in development at which a cell loses the chromosomal segment is read from the location and size of the "mutated" sector of recessive phenotype.

The next important point is that the "mutation" at the *Ds* locus (and a group of others studied) is controlled by another dominant, *Ac* (from "activation"), which must be present if *Ds* is to "mutate." *Ac* has no influence upon the normal *ds* locus, but similar events may occur at the *Ac* locus as at the *Ds* locus. (Comparable or different conditions at other mutable loci are not mentioned here.)

At this point, McClintock introduces another concept, the *state* of the locus. The state of *Ac* controls the time in development at which the "mutation" occurs at *Ds* and the state of *Ds* controls the frequency and location of the dissociation mutation. In other words, the mosaic pattern of mutation reflects the state of the loci, *Ac* and *Ds*, and the pattern usually remains constant throughout the life of the plant, but may also change, "mutate," at the time of cell division into one of two different subpatterns: complete reversion or an intermediate effect. This "state" is clearly a genetic property of the locus as it is typical for all descendants of a single plant in crosses, and in addition, remains largely constant in the offspring of a plant of definite type of variegation. Furthermore, not only the *Ds* locus but also the *Ac* locus has such states. The same is true for the recessive mutable loci, not mentioned thus far, which in the presence of *Ac* mutate to the dominant, all other phenotypical features being identical with those described for *Ds*.

One of these features is that the effect takes place not only quite late in the development of the sporophytic tissues, as is usual, but also at all stages in the development of the endosperm.

The next step in the analysis shows that time and frequency of the mutations are dependent on the *dosage* of *Ac*, of which 0–4 doses can be compared in the sporophytic tissue and 0–6 in the endosperm. The higher the dosage, the later the occurrence of the mutation. This applies to all mutable loci controlled by *Ac*. But there is a further complication: changes in the "state" of *Ac* also result in a time shift of the occurrence of mutation, which may be in the same or in the opposite direction as the dosage effect. An analysis could be made keeping the mutable locus constant (the same state) and considering different dosages as well as different states of *Ac*. It can be predicted which effect should occur in different combinations of dosage and state. The results can be explained by the assumption that the "states" of *Ac* are actually quantities: the *Ac* locus is supposed to be composed of a number of sub-units which can increase and decrease as a consequence of what may be called mutation within the locus. This conclusion is based upon the analysis of the characteristic pattern of sectors in kernels.

Further important facts were found for the recessive mutable loci *c* (aleurone color) and *wx* (waxy), which, in the presence of *Ac* show the "mutation" to the dominant condition, with otherwise the same details as described for *Ds*. Both *C* and *Wx* loci are also known to exhibit simple dosage effects. One mutable *c* produces always the dominant *C* effect. Another one can produce different intermediate conditions in addition to the full effect (multiple alleles). The same is true for the mutable *wx* locus. The analysis of the facts points to the assumption that these loci consist also of a series of sub-units and that "mutation" consists in increases (graded or maximal) in the number of these units in a depleted (*i.e.*, recessive) locus,

the visible effect being proportional to the number of units (plus threshold conditions). The different "states" thus resolve themselves also into quantitative differences in the units. The presence of twin spots showing different types of reciprocal behavior, *e.g.*, reciprocal conditions with reference to the time of occurrence of events at the *Ac*-controlled mutable loci, suggests that an unequal distribution of the units occurs at one mitosis, with one cell receiving too many, the other too few of them.

The last group of remarkable facts relates to the origin of the mutable loci. It was found that a submicroscopic piece of the chromosome containing the *Ds* locus can be transposed to a new location. This is indicated by the changed position of the subsequent break. If this new location is at or near the *C* locus the action of *c* is inhibited so as to act as if it were the recessive mutant *c*, but, in addition, this apparent *c* is now a mutable locus changing to *C* in the way reported before. This mutable change to *C* is assumed to be the consequence of removal of *Ds* by another transposition, thus restoring the normal *C* chromosome. There exist different states of *Ds* which lead to a more or less frequent removal and thus mutation to *C;* from this it is concluded as already indicated that *Ds* also consists of a number of smaller units and that the different states correspond to different numbers. These conclusions are borne out by the detailed facts.

We reported above that also *Ac,* needed for *Ds* action, is mutable in so far as different states may appear which influence the time of occurrence of *Ds* mutations. As comparable quantitative effects are produced by different doses of *Ac* it is concluded that the mutations of *Ac* consist in an increase or decrease of the number of sub-units at this locus, which otherwise behaves as a single Mendelian dominant. This *Ac* locus is, in addition, subject to transposition to other places and the different *Ac*'s produce their typical dosage effects even if they are located at different loci after transposition. The method of proving all this is rather

complicated. In the paper of 1950, *Ac* is considered to be heterochromatic and different quantities of heterochromatin are equated to the states.

2

The first general topic for our discussion is taken from that part of McClintock's work which deals with dosage experiments, the different "states" of the *Ac* and *Ds* loci and the relation of both of these to the phenotypic effect. It is this group of facts which has an amazing counterpart in the analysis of intersexuality in *Lymantria dispar,* thus showing that an apparently unusual genetic situation in an animal is also found in a plant in spite of the great differences of the two developmental systems. The main points of the maize case which come into play here are as follows: (1) the action of *Ac* shows a dosage effect so that the phenotypic effect (*Ds*-mutation) occurs later in development with increasing dosage. (2) *Ac* (and *Ds*) though behaving like simple loci in the Mendelian sense exist in different conditions, called states, which also control different times of incidence and intensities of the effect. (3) Dosage and state effects are to a certain extent interchangeable and collaborating, *e.g.,* high dosage plus low state act more or less like low dosage plus high state. (4) From this, McClintock concludes that the states are quantitative features of the locus, namely, different numbers of sub-units.

Let us now compare this with the facts of the Lymantria case. Here, the balance of the sex factors is the genic agent which controls the phenotype. But as in a given set of experiments the female factor or factors, located in the Y-chromosome, are constant, the different results, namely, normal sexes or intersexes of different degrees, are produced by the variants of the male determiners in the X-chromosome. Therefore, for our present purposes, we may neglect the constant female factors in the Y-chromosome. The dosage relation, paralleling that in maize, is produced here by the X-chromosome mechanism, which makes it possible

to compare one and two dosages of the male factor or factors. (In triploid intersexuality other dosages may be studied.) Clearly, one dosage of X, *i.e.*, of M (the male factor or factors) has a small action, too small to compete with the female factor or factors (F), and femaleness is the result. Two doses of M have a sufficiently strong action to produce maleness. Within this frame of basic dosage relations both F and M are found in different "states" which permitted the analysis. The "states" which were encountered in different natural races, are constant and genetic in a given race and therefore must have originated by mutation. We did not use the term state but spoke of different potencies, because the phenotypic effect is the increase or decrease of maleness (so-called female or male intersexuality) proportional to the potency, or the higher or lower state of the sex factors. This works as follows: If the potencies or states of F and M are in normal balance only the dosage relations of $1X = 1M = ♀$, $2X = 2M = ♂$ decide the result. If the potencies are unbalanced, the dosage effect can be overridden by the potencies or states so that with a low potency of F (found in the so-called weak races) one dose of $X = M$ may produce a male or any grade of intersex between female and male according to the potencies or states of M. *Vice versa* in the case of high potency of F (from a "strong" race) two doses of M (2X) of low potency or state will produce a female or any grade of intersexuality between a male or female, according to the respective potencies or states of M. (Heterogametic = 1X-intersexes, called female intersexes, and homogametic or 2X-intersexes, called male intersexes, are phenotypically different.)

The most elegant demonstration of the interplay of dosage and potency (state) is contained in a set of experiments in which a strong (high potency) F is combined with one M (one X-chromosome) from the same strong race and the other X (in a 2X individual = genetic male) is replaced by an X known to contain M of different potencies (see Goldschmidt, 1930). If the replaced M is in a sufficiently

high "state," a male is formed according to dosage 2X = ♂. If the replaced M is a little lower in potency the result is a low-grade male intersex. With decreasing potency of the replaced M the grade of intersexuality increases. If finally both M's are replaced by those of lowest potency, the dosage effect is completely overridden and in spite of the presence of 2X a female is produced.[1] The complete parallelism of these facts—of course minus the special feature of the sex-balance—with those found by McClintock is evident from the following description for the maize case (McClintock, 1948, p. 161):

> In an *Ac Ac ac* constitution (two doses) a low state *Ac* locus may give mutation patterns resembling that obtained from a high-state *Ac ac ac* (one dose) constitution. Again, the *Ac Ac Ac* (three doses) constitution of a low state *Ac* locus may give mutation patterns resembling those produced by a high state *Ac Ac ac* (two doses) constitution, etc.

But the parallelism goes still further. In Lymantria it was shown that the effect of the abnormal balances, the result of dosage and potency, is expressed in a happening, the so-called turning point at which, in intersexuality, female development changes into male development (see below). The position in time of this turning point is controlled by the relative potency or state of M or M M with constant F so that the switch-over reaction takes place earlier and earlier in proportion to the decrease of potency of M M in the case of strong F (male intersexes) and in proportion to the increase of the state of M in the case of a weak F (female intersexes). In the case of Lymantria with only 1X–2X available, the same can not be shown for different dosages. But in triploid intersexes of Drosophila where Dobzhansky (1930) found the same effects upon development dosage comes also into play.

[1] The present author takes this occasion to mention with great regret that the half million specimens of his Lymantria experiments left, together with the records, in the Kaiser Wilhelm Institute in Berlin, have been destroyed during the conquest of Berlin, when the boxes were smashed to steal the valuable glass lids. Thus only a very small set of representative specimens still exists, which was given to the Genetics Division of the University of California.

Exactly the same relations were found by McClintock in maize, discounting, of course, the differences in regard to the genetic basis. In this case the effect is the appearance of the somatic mutation. Whatever it may be in the individual case, it is found that the higher the dosage, the later in development the effect in the genetic and developmental system of the maize kernel. In the same way the higher the state of the individual *Ac* the later the effect, and both dosage and state show the quantitative interplay in so far as high dosage with low state will have an earlier effect than low dosage in the high state. Altogether, the facts are strictly comparable in spite of the completely different developmental system of the two organisms, which points toward a very general feature of the genic material being involved in both cases. Actually these facts found in Lymantria had been used to derive generalizations on the action of the genes which now are again being found in the maize case.

3

It is not surprising, under these circumstances, that the type of interpretation given to these facts had to be rather similar in both cases. In both instances, it was unavoidably concluded that the potencies or states represent different quantities of the genetic material. In the case of Lymantria the analysis was made at a time when no doubt in the reality of the classical corpuscular gene existed. Thus, in order to explain the results on the unavoidable quantitative basis the idea was developed (Goldschmidt, 1915, 1920) that a gene may change, by mutation, into a gene of different quantity, actually considered as a definite number of molecules and that these respective quantities control developmental reactions of proportional speed. This idea was subsequently (1927) elaborated into a detailed theory of genic action. In the present case of maize the classic theory of the gene has already become shaky and therefore a more radical quantitative solution has been proposed by McClintock. She concludes, "that the state of

any one *Ac* locus is an expression of the number of reduplicate units present within this locus." (The states are produced by mutation as reported above.)

Orthodox geneticists, who, in the Lymantria case were unwilling to accept the idea of potency or quantity of a gene, took refuge in the assumption that the M factor in Lymantria which behaved genetically as a single unit (also F), consisted, in fact, of a number of linked genes. The experimental facts would have required the corollary that these multiple genes were identical polygenes with an action proportional to their number. (I do not remember having seen this interpretation; but it was pointed out by some critics that the only exception found in innumerable experiments, two intersexes where none were expected, was an indication of crossing over.) There is, in Lymantria, unfortunately, no genetic or cytological possibility of distinguishing among the following possibilities: (1) a number of different, linked sex genes in the X-chromosomes (which does not account for the uniform quantitative results), (2) a block of polygenes which might be composed of different numbers in different races, (3) a single gene of different quantities (which is less probable today than it was thirty years ago), (4) a string of sub-units, differing in number, within a section of a chromosome which behaves as a Mendelian unit. In view of the great parallelism emphasized, the last, McClintock's interpretation of the *Ac* locus, may be the probable one, also, for Lymantria. There is a further parallelism. McClintock assumes *Ac* to be heterochromatic. I have discussed elsewhere the data (1948) which point to the heterochromatic nature of sex-determining sections.

4

A further link to recent developments in other fields can be forged if we consider the facts which are becoming more and more frequently found, facts which point to a general structure of small sections of the chromosome which behave as units, though they can not be identified with the classical gene. I have repeatedly discussed these facts in recent

years, and it seems that the factual material is constantly increasing. In a few words (see more detailed discussion in Goldschmidt, 1944, 1946, 1950) we may state the essentials thus: The smallest chromosomal sector which acts like a Mendelizing locus, is still subdivided. In the salivary gland chromosomes, it consists of a number of bands, the maximum number not yet being known. The phenotypic effect of any mutant in this section is the same or nearly the same, and this includes also position effects by breaks within the section. All these effects are also allelomorphic to each other. If mutants within this section can be separated by rare crossing-over the phenomenon erroneously termed "repeats" is obtained. This opens also the possibility that more than one part within the section mutates. One should expect such mutants to be additive and, therefore, an entire section would appear to be composed of subunits which control an action proportional to their numbers. A block of Mather's polygenes or a locus with quantitatively acting sub-units of McClintock or a "gene" with different potencies would be the result. It is furthermore possible that the action of the sub-units may be different in different substrates so that a differential action of the whole locus upon different organs appears in the way described by Stadler (1946) and D. Lewis (1949). If we take all these groups of facts together it seems that a very consistent idea of a genetic locus is emerging.

5

There are other facts which have been known for a long time without ever having found their proper place within the Mendelian concepts and which now fall in line with the new data under discussion. In 1919 Correns described the strange genetic behavior of a mutant of *Capsella bursa pastoris, albovariabilis,* which shows a highly variable mosaicism of white and green patches. Crosses with the type form show a simple Mendelian difference. But it is possible to shift the type of the homozygous mutant by selection from almost solid green to almost white. As this selection works also with branches of the same plant, ge-

netic modifiers are excluded and a kind of mutable gene must be assumed. But selection can be made repeatedly forward and backward so that ordinary mutation is excluded; only the completely normal type can be made constant by selection. Correns considered that the underlying gene was sick, which, within different cells of an individual, may be more or less sick and even normal. In trying to formulate an explanation in detail he realized at that time that some quantitative feature must be involved. This idea resembles McClintock's "state" or my quantity of the gene. Indeed, he even used the term "state." He assumed that the number of side chains in the gene molecule were variable and that during development they increased or decreased in number or even reached the normal maximum. If the grade of mosaicism corresponded to the number of these side chains, the results could be explained. This is clearly a somewhat crude expression of the ideas under discussion.

Still more interesting is the work of Lilienfeld (1929) which is almost unknown to geneticists, probably because the facts do not fit into the classic scheme. There is a dominant mutant of *Malva parviflora* with deeply laciniated leaves, sometimes even reduced to a single needle. In F_2 of a cross with the normal form a simple Mendelian 1:2:1 ratio is obtained. The homozygous *laciniata* are dwarfed and semilethal and die under ordinary conditions. If raised carefully they produce less laciniated leaves, even some almost normal looking ones. Sometimes out of the axil of a laciniate leaf a healthy branch sprouts with normal looking leaves, which practically hide the original *laciniata* stem. The new leaf form, though resembling the normal leaf, can be distinguished from it by some typical features; actually it is even more normal than the standard leaf. In 2–3 generations this new form is stabilized. If crossed now to the typical *M. parviflora* a simple monohybrid segregation is obtained, showing that the original monofactorial difference is still present. If a typical homozygous *laciniata* is selfed, using *laciniata* branches, it breeds true but for a few aberrant forms, among them also the

more than normal form just described. In the same way the once "stabilized" extracted hypernormal form breeds true. This same type crossed to real *laciniata* from which it sprang produces an F_1 of the same hypernormal type, which breeds true in F_2–F_5. Lilienfeld explains these facts in similar terms to Correns' explanation; the *laciniata* gene exists in varying states, corresponding to the almost completely reduced up to the more than normal phenotype. In ontogeny these states can change. Once the highest state is reached it remains constant and in addition transforms the low state in a heterozygote into the high one, so that the high-low hybrid breeds true for high.

This short review shows that another body of facts is here available which fits completely into the general quantitative picture of genic sub-units developed in the maize and Lymantria mosaics. The Malva case is especially important because it does not involve the simple alternative of chlorophyll or color production but such a complicated morphological feature as leaf form. The many details given by Lilienfeld should be analyzed by a plant morphologist from the standpoint of phenogenetics. Clearly the "states" or genic or subgenic quantities are seen to be acting in a parallel way upon color production, upon leaf shape and organization and upon sexual differentiation. Most probably also the time relations discussed before will be found to obtain also in the determination of leaf growth.

6

Once more I should like to return to intersexuality. The reason is that, in addition to the facts discussed above, mosaicism of a kind closely resembling that in maize (*mutatis mutandis*) is observed and has not yet found a proper explanation in spite of many discussions. In view of the parallelism of some of the basic facts and interpretations, the question arises whether the maize work might also help in explaining one of the most difficult phenotypic features of intersexuality in moths. The facts are these: In Lymantria female (one X) intersexes, as a rule, the male dark wing color appears all over the wing from the

lowest to the highest grades of intersexuality. But always in the intersexual males (2X) female color appears in large patches of a size increasing with the degree of intersexuality. In the white female patches size, shape and arrangement of the scales and also growth are of the female type while in the male sectors everything is of the male type. Thus a real mosaic is present just as if this organ belonged to a gynandromorph and not an intersex. If female intersexuality is produced by crosses with a certain race (Gifu) the same type of mosaic wings appear as in intersexual males, showing that the production of the mosaic is genetically controlled. These wing mosaics are of a type which can be compared with a maize kernel in which a few large colored sectors alternate with non-colored ones. In addition, a type of mosaicism can occur in intersexual females of all degrees in which tiny and numerous splashes of female scales are present on the background of male ones. Such splashed wings appear here and there without any known rule, probably caused by genetic modifiers. The type may be compared to a finely mottled (in small stripes) kernel (see pictures, 1912, pl. 1; 1920, pl. 2–5). In addition to these wing mosaics other intersexual organs may or may not exhibit a coarse mosaic structure in so far as a part of an organ, say one egg tubule, does not show the intersexual transformation of the rest.

There is one completely different type of intersexual mosaic known, which has been found only in one single genetic constitution (a cross with a Russian race) by Kosminski (see literature and analysis in Goldschmidt, 1938), where many male organs, like the antennae, exhibit mosaic structure, with female and male parts mixed in proportion to the degree of intersexuality. Sexual mosaicism also plays a considerable role in the triploid intersexes of a psychid moth, Solenobia, studied by Seiler, who was so impressed with the mosaic effects that he claims that the time law of intersexuality does not apply to these intersexes. I believe to have shown point by point that the time law (the turning point at different times) applies also to these intersexes when the decisive features are put in the foreground (see

Seiler, 1950; Goldschmidt, 1949) but that an additional mosaicism requires further explanation of a phenogenetic type. In this case the most conspicuous organs are the gonoducts which in intersexes show a mosaic of female patches with flat epithelium and male patches with high epithelium, the male patches increasing with the degree of intersexuality towards maleness. Altogether this epithelium, if spread out into a sheet, would exhibit the same mosaicism as the Lymantria wings just described, or some maize kernels.

An explanation of these mosaic structures must clearly contain the same elements as found in the maize case. They are as follows: (1) a time element, the time at which it is determined that a cell and its descendants will follow either the female or male developmental pattern. This time is the so-called turning point controlled by the balance of the sex factors. In a maize kernel the time is the occurrence of the "somatic mutation" controlled by the "states" of the loci involved. (2) A growth element is involved in so far as size and arrangement of spots in the mosaic depend both upon the time of occurrence of the switch-over reaction and the location and number of cells in which it occurs, as well as the number of subsequent cell divisions. This is again true both for intersexual and aleurone color mosaics. (3) The general developmental features of the organ will influence the pattern: in the wing the production of its typical shape and the location of the veins (analysis by my student, Minami, 1925), in the kernel its three-dimensional growth.

7

In view of all these phenogenetic parallelisms the question arises whether the genetic basis in maize, in spite of its complete diversity, can throw any light upon the genetic control of the mosaic development in the organs of intersexes which show it. However, the strictly alternative nature of the effect should first be emphasized. In maize the locus *c* may either "mutate" to *C* or may not. In Lymantria and Solenobia the wing or gonoduct cell may

either remain female or become male. There are no intermediate conditions existing. The decision of the alternative in maize is controlled either by the production of a chromosomal break resulting in a deficiency; or by a transposition which inhibits the action of the locus in the manner of a position effect (see below). Is it possible that something comparable produces the intersexual mosaic?

We have seen the alternative condition that exists in the wing of Lymantria. Whether it is the no-mosaic condition, *i.e.*, all cells with change of sex, or the mosaic condition, *i.e.*, only some cells with change of sex, depends upon at least two factors. One is the general genetic background of the individual which involves definite diversities in the mode of development. This appears in the obligatory mosaicism of all intersexual 2X individuals. The other is found in a special genetic control as evidenced in one X-mosaicism in Gifu crosses or in the presence of modifiers in those cases occasionally encountered but not analyzed further. Only in the special and peculiar case studied by Kosminski could I use the available data for a genetic analysis (Goldschmidt, 1938) of the specific modifiers.

In weighing these facts two types of explanation offer themselves. One is a phenogenetic explanation (though the phenogenetic processes are, as we saw, controlled or shifted by genetic modifiers or dosage systems). This means that all cells which must decide the female-male alternative are genetically alike. The decision occurring locally in some cells, and therefore leading to a mosaic, is brought about by a process which acts near a narrow threshold. Therefore, it is made in an all or none way where small local variations in the condition of a cell may be favorable or not. In other words, the unbalance of the sex factors produces in each cell at a certain time the switch-over reaction, *i.e.*, change of determination to the opposite sex. But it is successful only when a certain threshold is passed and it is the small individual variations between cells which decide whether it can be passed or not. In genetic language one might say that the effect is not completely penetrant. The genetic modifiers for mosaicism would affect threshold or pene-

trance. Other comparable phenogenetic explanations could be worked out as has been done repeatedly, *e.g.*, Goldschmidt (1923).

Though such a phenogenetic interpretation of the sex mosaics appears to be in order, the work on mutable loci raises the question whether, after all, a genetic explanation of the type found in maize could also account for the results superimposed upon the time law, controlled by the sexual balance. This would mean that the mosaic is not a purely developmental feature, but that at the time of the turning point something happens, in those organs which show mosaicism, which is comparable to the happenings at a mutable locus. One such possibility which is realized in maize might be that an individual cell division at the time of the turning point is unequal for the sub-units of the sex-determining locus so that one cell receives the number needed for male, the other that for female determination. If this were the case one could hardly understand why not all intermediate combinations should be produced, which is not the case though some facts in Lymantria might be made to conform to such an explanation. Another possibility might be a transposition as in maize, inhibiting the function of the locus near it. In the case of moths this would have to mean a transposition of something to the neighborhood of the female or the male determiners (in 1X- or 2X-intersexuality). It seems rather difficult to work out in detail such a scheme, though it might be accomplished by unequal crossing over between X and Y in the female. However, this would not work in the male. A third possibility might be that, similar to the action of the *Ds* locus, the turning point would produce in the development of organs exhibiting mosaicism an elimination of chromosomal material containing the sex determiners. This could obviously work only in the 2X = male sex. But we saw that sometimes also the female wings show this mosaic, which then would require a different explanation, *e.g.*, via non-disjunction. All this does not appear very likely so

that a phenogenetic explanation of the intersexual mosaics of the type mentioned above appears more probable, at least at the present moment.

8

From the point of view of basic genetic conceptions the most remarkable of McClintock's results is that mutation to a so-called mutable locus is the consequence of an insertion of the material composing *Ds* at or near the locus which becomes mutable. This means that the locus, called mutable, is not changed at all, but that the presence of the transposed *Ds* impedes its actions so that it behaves like its recessive. Removal of *Ds* in a cell during development restores the normal action. This looks like a return mutation to the dominant condition. It is obvious that the first part, the production of the recessive effect by insertion of *Ds*, is a typical position effect, a conclusion which McClintock also draws in her last paper. Also in Drosophila a break by translocation, deficiency, duplication, inversion can make a normal locus adjacent to the break act like its recessive (*e.g.*, scute, yellow). But there is also a parallel to the second part of McClintock's explanation available in Drosophila work, again emphasized in the latest paper; if the break near a given locus brings heterochromatin adjacent to the locus the position effect is of a mosaic nature. It should be realized that this can mean only three things: (1) Either the break and heterochromatic neighborhood makes the locus mutable, *i.e.*, mutating to its recessive only in some cells, as Schultz assumed. (2) The result is a typical position effect with recessive expression of the locus because of the break, but with an effect near a threshold of action (incomplete penetrance) which makes the effect take place irregularly here or there according to its passing or not passing the threshold in individual cells. I have favored this explanation thus far in Drosophila. (The irregularity of the effect does not apply to maize.) (3) In the light of McClintock's work, it is possible that in the course of de-

velopment the break is again removed by return inversion, etc., thus restoring the normal function in some cells and their derivatives leading to a mosaic. Thus far no facts are available to prove the last explanation. One should expect in case of its correctness that the return to normal could occur also in sex cells. Reinversions have been described by Grüneberg (1936) and Hinton (1950). But one must still explain why they are so rare in the germ track, and under the last hypothesis, so frequent in the organ affected by the locus. Thus we must wait to see whether this part of McClintock's analysis applies also to position effect mosaics in Drosophila. McClintock herself seems to assume now that the occurrence in time of position effect is controlled in Drosophila as in maize at least in part by the quantity of adjacent heterochromatin, thus accounting for the mosaic character of the effect.

One more fact should be mentioned in this connection which I have always considered to be full of hidden meaning. Gottschewski (1937) found in my laboratory that a Notch deficiency which genetically was clearly a deficiency had completely normal salivary chromosomes. More cases have been found since (Demerec, 1941; Barigozzi, 1942) with deficiencies of different length. The interpretation given by Gottschewski and accepted by his followers—actually only a circumscription of the facts—is that here the genes in question were inhibited. This would of course make the silent assumption that an inhibited gene acts like its complete absence, as all deficiency effects, N-phenotype and haplo-effect were present. I had always a feeling that a position effect of a hitherto unknown kind should be involved. McClintock's work suggests an explanation in such terms: an invisible transposition into this region after the manner of the *Ds* locus might inhibit the action of neighboring loci just as *C* is inhibited in the maize case by transposition of *Ds* adjacent to it. The extension of the effect to more than one locus is not a major obstacle to this explanation as position effects acting over a considerable distance, *i.e.*, affecting a number of loci, are already known for

breaks involving change of position of heterochromatin (see Demerec, 1941).

A discussion of problems of heterochromatin relevant for the present topic will be presented in a monograph on the podoptera effect in Drosophila, now in press.

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ERRATA

In the paper "Fifty Years of Genetics," p. 332 of this volume, last line, for "Jennings" read "Sonneborn."

ANOMALOUS SEGREGATION IN CROSSES OF *ESCHERICHIA COLI*

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INTRODUCTION

WHEN two diverse mutant lines of the sexually fertile bacterium *Escherichia coli* strain K–12 are crossed, the progeny exhibit extensive recombination of the genetic characters of the parents (Tatum and Lederberg, 1947; Lederberg, 1947, 1949, 1950*a*). This segregation resembles the corresponding phenomenon in higher organisms in that certain characters tend to be inherited in parental combination, but it is not such as to enable all the gene loci to be placed in a linear order by the usual methods (Lederberg, 1950*b*; Newcombe and Nyholm, 1950*a* and *b*). The present study deals with the nature of this anomaly.

MATERIALS AND METHODS

Lines W–677 and 58–161 of *E. coli* strain K–12 (supplied by Dr. Lederberg), and streptomycin resistant (S^r) and dependent (S^d) mutants derived from them (Newcombe and Nyholm, 1950*b*) were crossed. These lines possessed the following mutant characters:

W–677 *Lac*$^-$, *Mal*$^-$, *Xyl*$^-$, *Gal*$^-$, *Ara*$^-$, $V_1{}^r$, T^-, L^-, $B_1{}^-$; and,
58–161 B^-, M^-;

where the symbols represent lactose, maltose, xylose, galactose, and arabinose non-fermentation, bacteriophage T_1 resistance, and threonine, leucine, thiamin, biotin, and methionine requirement. S^r and S^d behave as alleles of a single locus (Demerec, 1950; Newcombe and Nyholm, 1950*a* and *b*), the wild type of which will be termed S^s.

Crosses were made by spreading washed suspensions of the two parents, (B^+, M^+, T^-, L^-, $B_1{}^-$, and B^-, M^-, T^+, L^+,

B_1^+) on minimal agar (Lederberg, 1947) supplemented with thiamin, and incubating the plates until growth factor recombinant (T^+, L^+, B^+, M^+) colonies appeared. These were categorized for phage resistance and streptomycin response by the cross-streak technique (Demerec and Fano, 1945), and for sugar fermentation by streaking on eosine-methylene blue agar plates to each of which had been added the sugar to be tested. (For details of the technique, and for the interpretation of segregation data where certain of the growth factor recombination classes are not recovered, see Lederberg, 1947.)

Results

Two crosses, (A) W–677 $S^s \times$ 58–161 S^r, and (B) W–677 $S^d \times$ 58–161 S^r, were made both in the absence of streptomycin and in its presence. (A concentration of 1000 units per ml. was used, and when one of the parents was S^s the streptomycin was sprayed onto the plate following a preliminary incubation during which crossing could be initiated.) When streptomycin was absent, both S^s and S^r colonies arose from cross A, and only S^r's from cross B; when it was present, cross A yielded only S^r colonies, and cross B both S^d's and S^r's. These selective environments (the presence of streptomycin in cross A and its absence in cross B) served to provide increased numbers of otherwise rare S^r's.

The numerical distributions of the various types of recombinants from the two crosses is shown in Tables 1 and 2. It will be noted that selection of S^r descendants is essentially the same whether accomplished by the presence of streptomycin or by its absence. (V_1 has been omitted from these tables but will be considered later.)

To test for genetic linkage, interactions have been calculated for each pair of characters in the conventional manner (see Tables 3 and 4). These show that *S* is linked to *Gal*, *Ara*, *Xyl*, and *Mal*, but not to *Lac* or V_1; that all sugar loci are at least loosely linked to all others; and that *Gal* is closely linked to *Ara*, and *Xyl* to *Mal*. V_1 is linked only to *Lac* (data not shown).

TABLE 1

Segregation in Cross A

(W-677 S^s × 58-161 S^r)

Recombination Classes				Recombinant Colonies							
				Unselected[1]				Selected[1]		Total	
				S^s		S^r		S^r			
Mal	*Xyl*	*Gal*	*Ara*	*Lac*+	*Lac*−	*Lac*+	*Lac*−	*Lac*+	*Lac*−	S^s	S^r
−	−	−	−	712	1,370	14	33	63	150	2,082	260
+	−	−	−	13	14	4	8	83	156	27	251
−	+	−	−	5	10	2	2	10	36	15	50
−	−	+	−	11	9	0	3	7	17	19	27
−	−	−	+	28	38	3	1	4	20	66	28
+	+	−	−	0	5	2	2	38	78	5	120
+	−	+	−	0	0	1	0	7	28	0	36
+	−	−	+	0	2	0	0	8	19	2	27
−	+	+	−	0	0	1	1	1	5	0	8
−	+	−	+	0	0	0	0	4	5	0	9
−	−	+	+	12	20	0	2	41	68	32	111
+	+	+	−	0	0	0	1	7	14	0	22
+	+	−	+	0	0	1	0	10	10	0	21
+	−	+	+	1	1	3	2	43	76	2	124
−	+	+	+	0	2	2	1	10	24	2	37
+	+	+	+	0	1	2	5	77	71	1	155
Total				782	1,471	35	61	443	777	2,253	1,286

[1]Unselected = streptomycin absent; selected = streptomycin present.

Discussion

Linearity of the gene array has not been conclusively established in *E. coli*. It will be assumed, however, in the following discussion.

On the basis of recombination frequencies with *S* the loci studied fall into the sequence: *S–Mal–Xyl–Gal–Ara–BM–Lac–*V_1*–TL*. Within the group *S–Mal–Gal–Ara*, however, this may not represent the actual gene arrangement. Thus, although linkage between members of the group is in good agreement, the frequencies of recombination with *BM* would place them in a different order (*S–Ara–Gal–Mal–Xyl–BM*) and linkage with *Lac* does not confirm either sequence, since it is approximately the same for all loci except *S* (to which it is unlinked).

TABLE 2

Segregation in Cross B

(W-677 S^d × 58-161 S^r)

Recombination Classes				Recombinant Colonies							
				Unselected[1]				Selected[1]		Total	
				S^d		S^r		S^r			
Mal	*Xyl*	*Gal*	*Ara*	*Lac*+	*Lac*−	*Lac*+	*Lac*−	*Lac*+	*Lac*−	S^d	S^r
−	−	−	−	102	224	1	9	18	34	326	62
+	−	−	−	2	2	3	1	34	41	4	79
−	+	−	−	4	1	0	0	3	10	5	13
−	−	+	−	0	0	0	1	2	5	0	8
−	−	−	+	4	3	2	1	0	6	7	9
+	+	−	−	2	0	1	2	8	24	2	35
+	−	+	−	0	0	0	0	4	7	0	11
+	−	−	+	0	0	0	1	2	10	0	13
−	+	+	−	0	0	0	0	0	1	0	1
−	+	−	+	0	0	1	0	0	1	0	2
−	−	+	+	4	3	0	0	4	11	7	15
+	+	+	−	1	0	1	0	2	5	1	8
+	+	−	+	0	0	0	0	0	3	0	3
+	−	+	+	0	0	2	0	19	17	0	38
−	+	+	+	0	0	1	0	2	0	0	3
+	+	+	+	0	0	2	0	43	48	0	93
Total				119	233	14	15	141	223	352	393

[1]Unselected = streptomycin present; selected = streptomycin absent.

Another means of determining the sequence was therefore used, that is, by comparing the frequencies with which each one of a group of three loci recombines with respect to the other two. Unless double crossing-over occurs more frequently than expected, the middle locus can be identified as the one which undergoes such recombination least often. However, if there is an excess of double crossovers the test may yield a false sequence, and conflicting sequences may even be indicated where more than three loci are considered. (For example, considering four loci, *A, B, C,* and *D* where crossovers in the *AB* region have a strong tendency to occur together with crossovers in the *CD* region, then the sequences *ABC, BCD, ADB,* and *ADC* will be obtained from the four possible comparisons.)

When this test was applied to the present data two conflicting sequences, *Mal–S–Xyl–Ara–Gal* and *Mal–Gal–Ara–S–Xyl*, were obtained, and it seemed likely that double crossovers were in excess.

This implication would be supported by showing that for groups of three linked factors the product of the supposed double- and non-crossover frequencies exceeded that of the two single-crossover frequencies, whichever of the three possible sequences was assumed. Thus, where a and b are the frequencies of crossing-over in two adjacent regions, and the crossovers are independent, the product of the non- and

TABLE 3

Linkage of *S* with *MAL*, *XYL*, *GAL*, *ARA*, *LAC*, and V_1

(Data from Tables 1 and 2)

Pairs of Loci	Cross A (W-677 S^s ×58-161 S^r)		Cross B (W-677 S^d ×58-161 S^r)	
	Interaction[1]	χ^2*	Interaction[1]	χ^2*
S-Mal	85.4	1537.0	122.1	372.9
S-Xyl	47.4	752.8	28.9	1520.0
S-Gal	26.6	865.3	35.2	180.2
S-Ara	13.5	702.9	19.6	160.4
S-Lac	1.0	0.006	1.3	2.3
S-V_1	0.9	0.3	1.3	2.5

[1]Interaction equals the product of the two parental types divided by the product of the two non-parental types. Where the interaction is significantly greater than unity the factors will be spoken of as linked.
*From 2 × 2 tables. $\chi^2 = 6.6$ is significant at the 1 per cent. level.

double-crossover frequencies would be $(1-a-b+ab)ab$, and the product of the two single-crossover frequencies $(a-ab)\ (b-ab)$. These expressions are equal, but if double crossovers were x times as numerous as expected the former would exceed the latter by the amount $xab-ab$.

The factors *S*, *Mal*, *Xyl*, *Gal* and *Ara* have been considered in this manner and in all but one of the ten comparisons (namely, *Mal–Gal–Ara*) an excess of double crossing-over was indicated. Essentially similar treatment was also given to data from the following groups of loci: (1) *Lac* and each of the other sugar loci (present data), assuming

that these are on the opposite sides of *BM* and treating *BM* as the middle one of the three in all comparisons, (2) V_6, *Lac*, and V_1 (data from Lederberg, 1947), allowance being made for the fact that double crossovers survive only when there is a third crossover outside of this group but within the *BM–TL* region, and (3) *Lac*, V_1, and *T* (data of Rothfels, unpublished), assuming the two possible sequences for *T* and *L*. In all cases some excess of double crossing-over was indicated (significant in 1 and 3).

Two interpretations of this effect are possible: either the

TABLE 4

Linkage between *MAL*, *XYL*, *GAL*, *ARA*, and *LAC*

(Data from Table 1; Cross A, W-677 S^s × 58-161 S^r.)

Pairs of Loci	S^s Colonies		S^r Colonies	
	Interaction[1]	χ^2[1]	Interaction[1]	χ^2[1]
Mal–Xyl	25.0	70.6	3.0	71.2
Mal–Gal	3.6	2.9	1.5	13.1
Mal–Ara	3.3	4.8	1.4	9.1
Mal–Lac	1.1	2.3	1.4	7.2
Xyl–Gal	6.1	6.8	2.1	38.6
Xyl–Ara	3.1	2.0	2.2	42.9
Xyl–Lac	0.5	1.7	1.4	18.2
Gal–Ara	61.0	474.0	36.8	652.0
Gal–Lac	1.4	1.7	1.3	6.1
Ara–Lac	1.2	0.9	1.5	21.6

[1]Interactions and χ^2 calculated as in Table 3.

double crossovers are formed in excess (in which case the phenomenon would be termed *negative interference*), or else they are favored by selection, the single crossovers tending to be eliminated. The latter could occur if the factors under consideration were: (1) on a ring chromosome, (2) between two lethal genes on the same chromosome (*e.g.*, between growth factor deficiency loci such as *B* and *M* when the growth factors are not supplied), or (3) within an inverted region.

In the present case ring structure would only favor double crossovers when they straddled *BM* or *TL;* and the lethal gene mechanism would operate only in the regions between

B and *M* and between *T* and *L*. Nor could inversions account for the excess of double crossovers since if an appreciable proportion of the loci were involved recombination classes derived from multiple crossing-over would be expected to be much rarer than they are. It can be shown, for example, that if *Mal, Xyl, Gal* and *Ara* are in an inverted region, quadruple crossing-over must occur about ten times as often as expected from the frequency of double crossing-over. A discrepancy of similar magnitude arises if the loci are assumed to be distributed some in a normal and at least two in an inverted region; and if *S* and *Lac* are considered at the same time this discrepancy is even more striking.

It should be noted that a slight excess of double crossovers (*i.e.*, not exceeding either of the single crossover classes) is mathematically indistinguishable from an excess of non-crossovers such as might result from irregular pairing. The conflicting indications of sequence, however, imply too great an excess to be interpreted in this manner.

This "negative interference" between crossovers does not necessarily represent a qualitative difference between *E. coli* and higher organisms. It is possible that the commonly observed positive interference exists also in the present material but is operative over a shorter range, and that the negative interference in *E. coli* is in some way related to the negative chromatid interference (Beadle and Emerson, 1935; see also Huskins and Newcombe, 1941), and negative chiasma interference (Newcombe, 1941) observed in higher organisms. It should be noted that from the cytological evidence these two effects would seem to be most striking where the number of chiasmata in a cell is high and distance betwen chiasmata is small.

The anomalous segregations in the present crosses are thus susceptible to interpretation along conventional lines. This does not rule out the possibility of some quite exceptional chromosomal phenomenon, particularly in view of the as yet unexplained gene losses in *E. coli* heterozygotes (Lederberg, 1949, 1950*b*).

These aberrant heterozygotes may contain genes in the hetero-, homo-, or hemizygous states, and more than one such state can be represented in a single bacterium. Thus it seems that gene loss and gene recombination both occur in the formation of the "het" strains. Further, when these heterozygotes are allowed to segregate there is a second recombination followed by loss of any inviable segregants.

It is not known whether similar events occur in ordinary crosses (the zygote stage being reduced to a single cell) but if they do it would complicate the above interpretation. The following alternative possibilities have been considered: (*a*) crossing-over in only one division, and separately in two divisions as in the "hets"; (*b*) gene loss prior to the initial crossing-over, and subsequent to it; (*c*) terminal loss, and interstitial loss; and (*d*) loss affecting chromosome regions from one parent only, and from either parent. Suitable combinations of the above possibilities could account for an excess of double crossovers but not for an excess of quadruple crossover classes. This does not entirely rule out loss phenomena as the cause of the present anomaly, since the above alternatives are not exhaustive. It does indicate, however, that interpretation along these lines would involve special assumptions.

ACKNOWLEDGMENTS

It should be noted that a number of workers (J. Lederberg, L. L. Cavalli, and G. Allen) have observed the apparent excess of double crossovers involving the sugar loci (Lederberg, personal communication), and that the present study differs mainly in the use of streptomycin to obtain the rare recombination classes, on the basis of which some of the formal possibilities have been eliminated.

The authors are indebted to Dr. Joshua Lederberg for information concerning his unpublished results and for criticism of the manuscript, and to Dr. Klaus Rothfels, who carried out preliminary experiments on the selection of rare recombinants by the use of streptomycin and of various sugars.

SUMMARY

Segregation of the linked group of loci, *S, Gal, Ara, Xyl* and *Mal* in crosses of *E. coli* is such that these can not be arranged in linear sequence by the usual methods. Assuming that there is no great deviation from familiar chromosome behavior, this anomaly appears to be due to an excess of double crossovers too great to be attributable to irregularities in chromosome pairing, and not such as could result from a selective mechanism acting against single crossovers (*e.g.*, an inversion, a ring chromosome, or a combination of lethal genes). This negative interference in crossing-over seems to occur in all chromosome regions tested.

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EVIDENCE FOR HAPLOID INTERSEXUAL FEMALES IN HABROBRACON

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SEX-INTERGRADES may be divided into two primary groups, genetic mosaics and non-mosaics. The genetically mosaic sex-intergrades are, in general, male-female mosaics, gynanders. In the parasitic wasp *Habrobracon juglandis* (Ashmead) there has been found, in addition to gynanders, a unique type known as gynandroid, which is mosaic for two kinds of male tissue (Whiting, Greb and Speicher, 1934). Normal males in a given stock are of two phenotypically similar types differing genotypically in their sex alleles, *xa* and *xb*, for example. In the females, which are sex heterozygotes *xa/xb*, there is a complementary action between the two sex alleles, each contributing factors dominant over recessive male factors present in the other. In gynandroid male mosaics, because development of the two types of tissue is more or less non-autonomous, there is likewise a complementary action between *xa* and *xb*. Consequently certain structures of the external genitalia become reduplicated and often definitely feminized. Gynandroids were for a time erroneously called intersexes before their mosaic nature was understood and before the complementary type of sex-determination had been demonstrated for Habrobracon.

In non-mosaic sex-intergrades all tissues of the body, whether male or female in structure, contain the same set of genes or of chromosomes. Such sex-intergrades include aberrant types and also a great variety of organisms with the individuals functioning normally both as male and as female—monoecious and hermaphrodite plants and hermaphrodite species of animals.

Aberrant sex-intergrades, frequently sterile, found in species with sexes normally separate, are often referred to as

hermaphrodites. The term is used for sex-intergrades in man and in other vertebrates. Non-mosaic sex-intergrades in insects, however, are, in general, called intersexes. Intersexes, as defined by Goldschmidt (1931), belong to two different series, male and female. The former begins development as a male, then, after a turning-point, develops as a female. Consequently the first-formed structures are of male type, the later of female type. Just the reverse holds for the female intersex. The turning point may be early or late and is not necessarily simultaneous for all organs.

Male and female structures may develop simultaneously in some intersexes and such individuals have been called hermaphrodites, as, for example, in the work of Lebedeff (1939). In a study of intersexuality in *Drosophila virilis*, he shows that females homozygous for a third chromosome gene (ix^m/ix^m) may develop into various types ranging from female-like intersexes to sterile males, depending upon modifying factors. "The development of the male sexual system, however, does not interfere with that of the female, resulting in the development of hermaphrodites of various degrees. The gonads in all morphological types of intersexes start their development as ovaries.—In hermaphrodites after the occurrence of the 'turning point' ovaries are transformed into ovotestes."

It might be better if the distinction between hermaphroditism and intersexuality were made on the basis of normality *vs.* abnormality, for, as the word hermaphrodite is now used, aberrant sex-intergrades, especially in vertebrates, are lumped with many normally functioning molluscs and worms and with plants having perfect flowers. The term intersex might then be extended to include all aberrant non-mosaic sex-intergrades whether vertebrate or invertebrate.

Misunderstanding often arises from the use of the terms male and female intersex. A "male intersex" may be largely female in structure and a "female intersex" may resemble a male. For this reason the senior author has pre-

ferred to substitute the terms intersexual males and females for male and female intersexes. We may perhaps more conveniently speak of masculinized females or feminized males. A weakly or a strongly masculinized female is an intersex, but if the masculinization is very weak or very strong the specimen is less intersexual in appearance than if it is of moderate degree. A completely masculinized female, a sex-reversed female, is not an intersex but a male. Similarly, a weakly or a strongly feminized male is an intersex, but if the feminization is very weak or very strong the specimen is less intersexual than if it is of moderate degree. A completely feminized male, a sex-reversed male, is not an intersex but a female.

Weakly feminized "intersexual" males in Habrobracon are caused by the recessive gene gynoid, *gy* (Whiting, Greb and Speicher, 1934; Whiting, 1943*b*). At casual inspection they give the impression of being male posteriorly, female anteriorly, resembling a type of gynander, but this is simply because their shortened, feminized antennae and their male genitalia are more obvious than their relatively heavy abdominal sclerotization, feminized, and their large male ocelli. For the most part they are normal males in function and internal structure.

A strongly masculinized "intersexual" female of Habrobracon has been described and pictured (Whiting, 1946). It was male externally except for its reduced female genitalia. Internally the poison apparatus was somewhat reduced while ovaries and seminal receptacle were lacking. Its antennal color (heterozygous for lemon) showed it to be diploid. It developed from an egg which was heavily x-rayed and subsequently fertilized. The suggestion was made that a mutation may have been induced in its maternally derived sex-differentiating allele, partial inactivation of which would cause a shift toward maleness. This specimen was, in structure, as male-like as a gynoid male. Nevertheless, the very fact that organs appearing late in development, the male antennae, were present, while testes

were absent, places it in the female rather than the male series of intersexes. Otherwise, developing as it did from a fertilized egg, it might have been a diploid intersexual male.

Nine intersexes, all alike, were reported (Whiting, 1943*b*) among offspring of a single female which had been exposed to males. At casual inspection they suggested gynanders, male anteriorly, female posteriorly, reverse to the type suggested by gynoid. Head and thorax were male. Abdominal sclerotization was heavy posteriorly (female) becoming thinner anteriorly (male). Sting and sensory gonapophyses, poison sack and glands and seminal receptacle were normal for the female. Ovaries were present but small, each consisting of two sacks of oogonia with no differentiation of nurse cells or ova. Mating behavior of these intersexes was that characteristic of antero-posterior gynanders; active behavior was male, but males mated with them since they were passive in respect to receptivity to males. Since it was believed that they had developed from fertilized eggs and since the gonads were female, they were considered to be diploid intersexual females. The "mutation" in this case was spontaneous, from non-x-rayed stock. Further details are discussed below.

Recently (April, 1950) the junior author found thirty-four intersexes which appear to be similar in every way to the nine just described. The mother, however, was unmated, a daughter from the cross of a wild type (stock 33) female (x-rayed, 28,000 r, at the Hospital of the University of Pennsylvania) by an untreated ivory-eyed male (stock 17–o^i). These intersexes likewise had large male ocelli and long male antennae and abdomens largely female. Two of the intersexes and two of their twenty-eight brothers had asymmetrical antennae in that the right differed from the left by a single segment. Antennae were symmetrical in segment number in twenty-four of the intersexes and in twenty-two males. One or both antennae were broken in the remaining specimens.

Antennal segments are normally fewer and thicker in females than in males. However, reduction in number is not necessarily an indication of feminization. Many mutant types show reduction, irregularity or fusion of antennal segments in both sexes. The antennae of the thirty-four intersexes appeared to be as slender as those of their brothers. There were fifty-eight complete antennae of the intersexes ranging in flagellar segments from seventeen to twenty with frequencies seven, eleven, twenty-seven, thirteen, respectively, and there were forty-eight of their brothers ranging from seventeen to twenty with frequencies two, nine, twenty-one, sixteen. The distributions suggest that those of the intersexes may average slightly shorter, but the difference is not significant by chi-square test.

The usual sex types in Habrobracon, haploid males, diploid females and diploid males, have been shown by counts of wing microchaetae to differ in cell size. Comparison of the distribution of the microchaetae of these intersexes with that of their brothers (haploid) showed similarity indicating cell size to be at least approximately the same.

Examination of the internal structure of several of these intersexes showed them to be in every respect similar to those found in the lot of nine recorded previously (Whiting, 1943*b*). Some had evidently mated with their brothers as indicated by the presence of sperm in the seminal receptacles. The ovaries were small sacks filled with undifferentiated cells resembling oogonia. No dividing figures could be found, so that the haploid nature of these intersexes could not be verified cytologically.

As regards eye color, the sixty-two members of the fraternity were classified as intersexes—black twenty-nine, ivory five; males—black thirteen, ivory fifteen. It was suggested by the junior author that in the x-rayed egg from which the mother of this fraternity arose, there may have been a " translocation " involving an *x*-allele (*xf* of stock 33) which became linked with the dominant allele to ivory,

o^+. Fertilization by an untreated ivory-bearing sperm ($xg\ o^i$ of stock 17-ivory) would give a female of composition $\underline{xf\ o^+}/\underline{xg\ o^i}$, the mother of the fraternity under discussion. Sex types and eye colors of progeny resulting from various combinations of these genes are indicated in Table 1; *xg xf* in combination with a haploid set of other genes may give rise to intersexes, and *xg* or *xf*, combined with a haploid set of other genes, to males. Lack of an *x* factor may be lethal. The scheme here presented also accounts for the equality of black and ivory among the males and the excess of black among the intersexes. The daughters of males with the "translocated" *x* factor (italicized in Table 1) would be

TABLE 1

Types of Haploid Progeny Expected from Various Combinations of Genes in the Eggs Produced by an Unmated Female of Composition $\underline{xf\ o^+}/\underline{xg\ o^i}$ (from Mother) and $\underline{xg\ o^i}$ (from Father). *xf* Has Become Linked with o^+ by "Translocation." *xg* Assorts at Random with *xf*.

	Non-crossovers		Crossovers	
	$\underline{xf\ o^+}$	o^i	$\underline{xf\ o^i}$	o^+
xg	Black intersex	Ivory male	Ivory intersex	Black male
—	*Black male*	Inviable	*Ivory male*	Inviable

expected to produce intersexes. Unfortunately the males were killed before the hypothesis was formulated and tests made.

It is of interest to note that linkage with orange, *o*, an allele of ivory, was suggested in the fraternity of the nine similar intersexes reported previously (Whiting, 1943*b*). The black-eyed mother of these nine had eclosed with orange-eyed brothers. There were reported from vial a, females—black ten, orange three; males—black three, orange eight, and from vial b, intersexes—black seven, orange two; males—black four, orange nine. Because of their structure and because the mother had been exposed to males, these intersexes were at the time regarded as being derived from fertilized eggs, but it was difficult to understand why the

supposed zygotic offspring in the first vial should be females, in the second intersexes. The fraternity was one in a series which was being bred in order to separate the eye color pellucid from orange which it masks. The wasps in vial a may have died before being counted and the antennae may have been broken. Consequently the possibility must be recognized that the "females" were, in fact, intersexes and that the mother was unmated. On this basis a summary indicates: intersexes—black seventeen, orange five; males—black seven, orange seventeen. Although numbers are small, linkage with the orange locus is indicated as it is in the fraternity recently found. Should this mutation again occur it may be possible to continue the stock, obtaining further fraternities with intersexes in F_2 from some of the brothers and to analyze the cytogenetic conditions.

In a discussion of the origin and maintenance of purely female, parthenogenetic races or species of Hymenoptera, the senior author suggested (Whiting, 1943*a*) the possibility of the production of haploid females. Females "homozygous" for *x* "might be produced if *x* were duplicated either by translocation or by unequal crossing over so that *xa/xb* + 2A would become *xa xb/xa xb* + 2A. Theoretically, haploid females *xa xb* + 1A would then be possible."

Although no chromosome counts could be made, the idea that the thirty-four intersexes recently found are haploid is supported by the facts that their mother had not mated, that their wing cells resembled those of their brothers and that females were not present in the fraternity. The idea that they are intersexual females rather than intersexual males is evidenced by their structure—the presence of ovaries—even though rudimentary—and of poison apparatus and female genitalia, while segmentation of the antennae, occurring late in development, after the turning-point, is male. This structure is reverse to that of the gynoid males with their testes, male genitalia and female antennae. That the thirty-four are intersexual rather than purely female may in some way be due to an upset in genic balance caused

by two different sex alleles in the haploid rather than the diploid set of chromosomes.

The problem of genic balance for sex determination in groups with haploid males must differ from that in groups showing sex determination of the more usual backcross type. In the latter any given sex-differentiating factor always has a similar influence. Thus in Drosophila X is always female-potent, Y is inert. The autosomes average toward male potency. Knowledge of this is based on aneuploidy involving entire chromosomes. In Habrobracon the male potency of any gixen *x* factor is negatived by the presence of a different *x* factor. Thus while *xa* or *xa/xa* and *xb* or *xb/xb* tend toward maleness, *xa/xb* tends toward femaleness. No information has been obtained regarding aneuploidy or the influence of autosomes or of regions of the sex chromosomes crossing over with *x*. Translocations and intrachromosomal changes involving *x* may account for "mutations" causing intersexuality but may complicate simple genic balance by reorganization of structure, "position effect."

Summary

Sex-intergrades are either genetically mosaic or non-mosaic. Among the latter redefinition of the words hermaphrodite and intersex is suggested. Relative advantages of the terms feminized male, intersexual male and male intersex as also of masculinized female, intersexual female and female intersex are discussed.

The occurrence is reported of thirty-four presumably haploid intersexual or masculinized sterile females in one fraternity produced by an unmated daughter of an x-rayed mother. Their structure and behavior are described and comparison is made with intersexes previously found in Habrobracon. Linkage of the "gene" causing this intersexuality with the locus for ivory eyes is indicated. It is suggested that the "mutation" may have been a translocation giving the possibility of haploids with two sex alleles—*xg xf* + 1A.

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SPONTANEOUS CHROMOSOME FRAGMENTATION IN *TRILLIUM ERECTUM* L.[1]

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INTRODUCTION

MANY studies have been made of spontaneous mutation rate in a variety of organisms, but relatively few quantitative studies of spontaneous chromosome aberrations have been reported. The present study of spontaneous aberration was undertaken in order to establish a "background" rate and with the hope that a better understanding of spontaneous breakage might throw some light on the mechanism of radiation-induced breakage, loss and rearrangement.

The frequency of spontaneous chromosome breakage or fragmentation in unirradiated *Trillium erectum* L. sometimes approaches or exceeds that found in low dosage radiation experiments. In order to establish whether a statistically significant change in aberration frequency has occurred it is usually necessary to count a very large number of cells. Since all the material required can not conveniently be obtained from a single plant, it is important to determine whether or not different plants used are homogeneous with respect to aberration frequency. It is also important to establish whether or not significant differences in break frequency exist between buds within a plant and between anthers within a bud.

Chromosome fragments or their derived micronuclei can be scored at several different stages during microsporogenesis. As a consequence it is often necessary to compare aberration frequency at different stages. For this reason comparisons of the numbers of aberrations present at each of four different stages have been made. Where no signifi-

[1] Research carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.

cant differences in aberration frequency exist between stages the values obtained may be used interchangeably in untreated material.

Materials and Methods

Plants were received[2] in October and stored at 4°–6° C. until used, as previously described by the authors (1948). All counts were made on permanent smears of microsporocytes or microspores stained with iron propiono-carmine.

To test for differences between plants, between buds within a plant and between anthers within a bud, fifteen two-budded plants were used, and smears made from two anthers of each bud. For determination of aberration frequency, counts of micronuclei were made at the microspore interphase. Approximately half the scoring on each slide was done by each of two assistants. The total number of cells scored is presented. No significant differences existed between the values found by the two scorers. Occasional rescoring also indicated a high degree of reproducibility.

Smears from eight plants (10 different flower buds) were used for comparing the aberration frequency of different stages of microsporogenesis. The stages compared were first anaphase, quartet, microspore interphase and microspore metaphase. From six buds, slides of all four stages were obtained. No quartets were obtained from the other four buds. Fragments were counted at first anaphase and microspore metaphase; micronuclei were counted at quartet and microspore interphase. Chromatid or chromosome fragments can occasionally be seen at first metaphase, but scoring at this stage is considered less reliable.

In the initial scoring for stage comparisons the data showed significant differences. The data presented in this paper were obtained by both scorers examining every field of cells scored and keeping independent records. Using this

[2] The plants were purchased from Gardens of the Blue Ridge, Ashford, N. C.

method identical counts were obtained for each slide in all cases.

A more detailed consideration of the statistical methods used will be given elsewhere (Brandt, unpublished).

Results

Statistical analyses of the frequency of micronuclei found in microspore interphase cells (method of A. E. Brandt, unpublished) showed that there was no significant difference between anthers within a bud or buds within a plant (Table I). However, the plants examined fell into two groups (Tables I and II). Of the fifteen plants used, eleven had a low mean aberration frequency of 0.666 per cent.

TABLE I

Analysis of Spontaneous Aberrations (Micronuclei) of (1) Anthers within a Bud, (2) Buds within a Plant and (3) Plants within a Group

	Anthers	Buds	Plants
	Signif.	Signif.	Signif.
All plants (15)	not	not	very
Low group (11)	not	not	not
High group (4)	not	not	not

There was no significant difference between plants within this group. The other four plants with a mean of 2.908 per cent. had a significantly higher number of micronuclei than the first group. Differences within this group also were not significant. As may be seen in Table II, there was no overlapping in percentage aberrations between group I (low aberration frequency) and group II (high aberration frequency). The plant with the highest percentage of micronuclei in group I (0.94) had half that of the lowest one of group II (1.88). In the cells scored only four cases were found with two aberrations per cell.

As there is normally no interkinesis in *T. erectum*, a fragment present at first anaphase persists into second division and appears as a micronucleus at quartet and microspore interphase. During this last stage they usually degenerate.

TABLE II

Summary of Data from 15 Plants Showing Frequency of Micronuclei Scored at the Microspore Interphase. (Except Where Noted, 400 Cells Were Scored from Each Anther)

	Plant number	First bud		Second bud		Total for Plant		
		First anther	Second anther	First anther	Second anther	Number of Micronuclei	Number of Cells	Percentage† of micronuclei
Group I	2,362	1	2	1	0	4	1,600	0.25
	2,199	0	3	1	2	6	1,600	0.38
	1,537	0	3	3	2	8	1,600	0.50
	2,303	1	7[a]	1	0	9	1,526	0.59
	2,027	0	1	7	2[b]	10	1,500	0.67
	2,384	5	1	0	5	11	1,600	0.69
	922	0	5[b]	0	5[b]	10	1,400	0.71
	2,177	2	4[d]	3	4	13	1,600	0.81
	2,274	2	5	2	4	13	1,600	0.81
	2,355*	4	4	3	2	21	2,400*	0.88
	2,355	...	...	3	5			
	2,173	1	4	4	6	15	1,600	0.94
	Subtotal					120	18,026	Mean = 0.666
Group II	2,221	5	15	8[e]	2	30	1,600	1.88
	2,309	12	12	11	9	44	1,600	2.75
	2,383	13[c]	18	11	12	54	1,562	3.46
	2,290	19	15	11	12	57	1,600	3.56
	Subtotal					185	6,362	Mean = 2.908

a, 326 cells only were scored, one had 2 micronuclei; b, 300 cells scored; c, 362 cells scored; d, one cell had 2 micronuclei; e, two cells had 2 micronuclei.

* Plant No. 2,355 had three buds, hence total of 2,400 cells were counted.

†Percentage calculated on basis of number of microspores (not on basis of number of quartets).

Fragments present at microspore metaphase are considered to be newly formed and not the same ones previously produced during meiosis.

The numbers of fragments observed at first anaphase and at microspore metaphase, and of micronuclei at quartet and microspore interphase are given in Table III. At each stage, 200 cells were counted (exceptions noted in footnote to Table III). However, it should be understood that each first anaphase or quartet produces four microspores and

TABLE III

Number of Aberrations Scored at Different Stages in Microsporogenesis in Eight Different Plants (10 Different Flower Buds) of *Trillium Erectum* L. (Only one Aberration per Cell Except as Noted in Footnotes)

Plant number	Stages Scored			
	First anaphase	Quartet	Microspore interphase	Microspore metaphase
	Fragments per 200 first anaphases	Micronuclei per 200 quartets	Micronuclei per 200* microspores	Fragments per 200* microspores
1,929	6	6	6	1
2,001	1[a]	2	2	1
2,127	2	5	3	1
2,173A	6[b]	...	0	1
2,173L	2	...	2	0
2,199A	3[d]	0	1	2
2,199L	1	2	2	1
2,217L	10	9[d]	4	0[c]
2,305	5[e]	...	3	0
2,423	1	...	0	0

a, 170 cells only; b, 189 cells only, one cell had 2 fragments; c, 174 cells only; d, one cell had 2 fragments; e, two cells had 2 fragments each.

* 200 microspores are derived from 50 first anaphases or 50 quartets. For direct comparisons the values of the last two columns must be multiplied by four.

therefore 200 microspores are equivalent to 50 first anaphases or quartets. A statistical analysis (unpublished method of A. E. Brandt) of the data in Table III showed that there were no significant differences in aberration frequency between first anaphase, quartet and microspore metaphase. However, microspore interphase gave significantly higher counts than the other three stages (Table IV).

Table V gives a comparison of aberrations found in these four stages based on all the material analyzed. However, in four of these buds the quartet stage was not represented. By using only the six buds in which all four stages were found an even more striking comparison was noted (Table VI). For the three stages showing similar aberration frequency the mean was approximately two per hundred microsporocytes (0.5 per 100 microspores) compared with six per hundred microsporocytes (1.5 per 100 microspores)

TABLE IV

Stage Comparisons of Aberration Frequencies During Microsporogenesis (Data of Table III)

Stages compared	Significance
Anaphase I—Quartet	Not significant
" —Microspore metaphase	" "
Quartet — " "	" "
Microspore interphase—Anaphase I	Very significant
" " —Quartet	" "
" " —Microspore metaphase	" "

when scoring was done at microspore interphase. The reason for the much higher value of micronuclei at the latter stage is not understood.

The microspore metaphase breaks do not represent the same aberrations already scored at earlier stages. It is therefore necessary to add the value for microspore metaphase to the meiotic breaks to get total aberration frequency. However, since two statistically significant values were obtained at earlier stages two values are possible. The minimum value obtained by adding first anaphase (or quartet) to microspore metaphase is four per hundred microsporocytes (one per 100 microspores) (Table VI). The maximum value would be obtained by adding microspore interphase and metaphase giving a total of eight per hundred microsporo-

TABLE V

Spontaneous Aberrations at Four Stages of Microsporogenesis in *Trillium Erectum* (Summary of Table III)

(Data from 10 different buds for all stages except quartet which was from 6 only)

Stage	Type of Aberration	Total no. of		Aberrations per 100 PMC (or 400 microspores)
		Cells	Aberrations	
Anaphase I	Fragments	1,959*	37	1.89
Quartet	Micronuclei	1,200	24	2.00
Microspore interphase	Micronuclei	2,000†	23	4.60
Microspore metaphase	Fragments	1,976†	7	1.42

* Microsporocytes (PMC) † Microspores

TABLE VI

Spontaneous Aberrations at Four Stages of Microsporogenesis in *Trillium Erectum*

(All 4 stages were scored in each of 6 different buds as indicated in Table III)

Stage scored	Type of Aberration	Total no. counted		Aberrations per 100 PMC (or 400 microspores)
		Cells	Aberrations	
Anaphase I	Fragments	1,170*	23	1.97
Quartet	Micronuclei	1,200*	24	2.00
Microspore interphase	Micronuclei	1,200†	18	6.00
Microspore metaphase	Fragments	1,166†	6	2.04

* Microsporocytes (PMC) † Microspores

cytes (two per 100 microspores). It is not known which of these values is the more accurate.

Discussion

Spontaneous chromosome aberrations in plants have been described by a large number of authors. In many cases the aberrations are associated with genetic or structural hybridity (Emsweller and Jones, 1938; Giles, 1940, 1941; Walters, 1950). In some cases they have been reported in normal plants (Nichols, 1941; Giles, 1941; Sax, 1941; Darlington and Upcott, 1941; Bhaduri, 1942) but no previous statistical study has been made of the frequency of spontaneous fragmentation at different stages of microsporogenesis in normal diploid plants. Emsweller and Jones (1938) presented a limited amount of data on frequency of fragmentation at various stages in a hybrid plant of *Allium cepa* × *A. fistulosum*. Differences were found between various stages, but no statement was made concerning the probability that they might have been differences occurring by chance in undifferentiated material.

In the present paper we have been concerned mainly with the frequency of acentric fragments (or micronuclei) not primarily associated with other types of structural or numerical chromosome changes. For a consideration of the origin and fate of spontaneous breaks and derived aberra-

tions the work of Emsweller and Jones (1938), Darlington and Upcott (1941) and Walters (1950) should be consulted.

The presence of spontaneously occurring fragments not associated with other types of aberrations complicates the interpretation of data from low dosage radiation experiments unless adequate control material is collected. The statistically significant difference between plants makes this even more essential than it would be in an inbred or clonal line. In the latter case individual plants of *Tradescantia paludosa* show insignificant differences in number of micronuclei at microspore interphase while non-clonal plants show greater variation (Sparrow, unpublished).

The frequency of micronuclei in our individual plants varied from a low of 0.25 to 3.56 per cent. (Table II). Other cases were noted where the aberration frequency was even higher, including one plant with a few cells showing an extremely high amount of fragmentation (Sparrow and Christensen, 1950). It is therefore obvious that control data should be collected whenever a low frequency of induced breakage is expected. The fact that first anaphase, quartet and microspore metaphase stages give non-significant differences in aberration frequency indicates that data collected from any one of these stages may be used as a control for any other in normal untreated material. Such counts can be obtained by removing an anther (or bud) before irradiation. These may be kept alive as excised organs until they reach a suitable stage for smearing by placing them in a moist chamber or in a culture nutrient such as Hoagland's. The latter is more satisfactory for periods greater than three or four days.

Fragmentation in somatic cells of untreated *Trillium erectum* has also been reported (Wilson and Sparrow, 1941; Bailey, 1949). However, no quantitative study has yet been made and it is not known whether the frequency in somatic cells is indicative of that in meiotic or post-meiotic stages. In *Tradescantia,* Giles (1941) found no correlation between spontaneous break frequency in root tip and microspore nuclei.

In *Trillium* lagging chromosomes are occasionally observed at first anaphase. These can readily be distinguished from chromatid fragments and were not included as such. However, lagging chromosomes at second anaphase would be expected to form micronuclei occasionally and these would not be distinguishable from micronuclei formed from acentric fragments. However, the fact that there is no significant difference between first anaphase and quartet counts indicates that lagging second anaphase chromosomes contribute a small or negligible proportion of subsequent micronuclei.

The difference in numbers of micronuclei between the quartet and interphase is not understood. Since each quartet yields four interphase cells it was expected that the number of micronuclei would be almost identical, or possibly smaller at interphase if some of the micronuclei had degenerated. The only plausible explanation would seem to be that additional micronuclei appear during interphase. Their origin is unknown, but it has been suggested (by M. J. Moses) that deficient chromosomes, *i.e.,* those which lost a portion as a fragment, may for some reason be unable to function normally in the haploid nucleus. They might therefore be passed out of the nucleus and appear during resting stage as additional micronuclei. If such a phenomenon happens it could account for a doubling of the number of micronuclei, but the data show a threefold increase. The origin of the remainder is not apparent and no satisfactory explanation is available. Further work with *Trillium* and *Tradescantia* is now under way to confirm this unexpected increase in number of micronuclei and to try to establish their origin. Preliminary results with *Tradescantia* indicate that the number of micronuclei increases during later interphase (R. DuBow, unpublished).

The genetic significance of spontaneous chromosome fragmentation is difficult to assess, but it seems probable that it is of considerable evolutionary importance since it can afford a mechanism for new structural rearrangements as well as loss or duplication of whole blocks of genes (Garber,

1944; see also McClintock, 1949). The small supernumerary chromosomes frequently found in *Trillium* (Sparrow and Pond, 1950) and other plants may originate by a combination of fragmentation and duplication. It is assumed that large deficiencies resulting from either terminal or interstitial deletion would act as dominant lethals and hence would not persist as "mutations" into the following generation (*cf.* Muller and Pontecorvo, 1942). Smaller deficiencies on the other hand might be perpetuated. In polyploids the situation would be somewhat different as pointed out by Huskins (1941) and others and chromosome aberrations would be expected to survive more frequently.

It has frequently been suggested that "spontaneous" chromosome fragmentation (and mutation) are the result of naturally occurring radiation. However, the results of Giles (1940) and Sparrow (1950) indicate that a very large increase over natural radiation is required to increase the aberration frequency significantly (see Catcheside (1948) and Hess and Eugster (1949) for a more detailed account of possible effects of natural radiation). It seems very doubtful therefore that this source of radiation is a major factor in the production of "spontaneous" chromosome breaks and subsequent rearrangements. Physiological or physical factors (other than radiation) are probably responsible. Genetic factors also seem, in certain cases, to be of some significance (Clark and Copeland, 1940; Nichols, 1941; Giles, 1940, 1941; Warmke, 1946; McClintock, 1949; Sparrow, unpublished). Up to the present time no really satisfactory explanation of the origin of spontaneous chromosome breaks has been put forward. However, it is hoped that further study of the factors controlling both natural and induced chromosome breakage may throw some light on the mechanism and origin of breakage and reunion.

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Summary

The frequency of spontaneous chromosome fragmentation has been studied in *Trillium erectum*. Fragments at first anaphase and microspore metaphase and micronuclei at quartet and microspore interphase were counted and treated statistically. Analysis of micronuclei at microspore interphase in fifteen plants showed no significant difference between buds within a plant or anthers within a bud. However, the fifteen plants fell into two groups with mean frequencies of 0.666 and 2.908 micronuclei per 100 microspores. There was no overlapping and the difference was statistically significant. It is concluded that "background" counts of spontaneous aberration should be made whenever accurate control values are needed in radiation experiments.

No significant difference was found between aberration frequency at first anaphase (1.97 per cent. fragments), quartet (2.00 per cent. micronuclei) and microspore metaphase (2.04 fragments per 100 PMC) when material was all collected from the same plants. However, microspore interphase had an aberration frequency of 6.00 per 100 PMC or about three times that of the other three stages. The reason for the significantly higher frequency at interphase is not understood. The results suggest that the values obtained at any one of three stages (first anaphase, quartet or microspore metaphase) can be used interchangeably as a control count of spontaneous fragmentation.

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THE RELATION OF SEX RATIO TO PHYSIOLOGICAL AGE IN THE WILD BROWN RAT[1]

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ALTHOUGH much work has been done concerning the sex ratios at birth and at various ages of many domesticated animal populations, little has been accomplished in this respect with animal populations in their natural state. The problem here involves the difficult task of collecting a sufficiently large number of specimens to be able to generalize from the data with a fair degree of confidence. King (1924) has determined the sex ratios at birth of large numbers of albino and of brown Norway rats in captivity; a summary of her data is presented in Table I. The great difference that she found between the sex ratios of the albino and the brown rats in captivity raises the question as to whether this reported sex ratio is also valid for brown rats in their normal habitat.

The object of this paper is to determine the sex ratio in a population of wild brown rats (*Rattus norvegicus*) living

TABLE I

Sex Ratio at Birth of *R. norvegicus* in Captivity (King, 1924)

Strain	No. of young	Per cent. male	S.D.[1]	P[2]
Albino	4,992	51.26	0.708	0.07
Wild brown	1,862	46.19	1.160	0.002
Cross between albino and brown	3,338	51.53	0.867	0.07

1 Standard deviation.
2 Probability that the observed difference from 50 per cent. could be obtained by chance.

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under natural conditions. In addition, an attempt was made to determine the sex ratios of various age segments of the population.

Methods

In recent years, this laboratory has collected the reproductive tracts from more than 2500 wild female Norway rats, of which 435 were visibly pregnant and, in addition, a series of uteri of albino rats. These albino rats were killed on successive days after copulation in order to determine the size of the embryo in relation to the number of days since copulation. These data are shown in Table II, where the length was determined by arranging the litter according

TABLE II

Relationship of Age to Crown-Rump Length in Albino Rat Embryos

Days since copulation	Length (mm.) of median embryo	Extremes of length (mm.)	Number of young measured
12.5	5		12
13.5	7	4 - 7	11
14.5	8	8 - 8	10
15.5	9	9 - 10	6
16.5	12	12 - 13	5
17.0	14	14 - 15	10
18.5	18	17 - 20	13
21.5	33	30 - 37	9

to size and measuring the crown-rump length of the median embryo; the "range of length" refers to the lengths of the smallest and of the largest embryos of the series, and the "number of young measured" refers to the size of the particular litter.

The smallest embryo from wild rats in which sex could be positively identified macroscopically was 15 mm. long, which meant that all embryos less than 17 days of embryonic age (Table II) were useless for the present objectives. Therefore, of the 435 pregnant uteri mentioned above, only 118 contained embryos large enough to be used.

To determine the sex of the embryo, a transverse incision

was made posterior to the navel, and the position and shape of the urogenital system was the determining factor. In the male, one testis lay on each side of the colon, and the ductus deferens looped ventrad to the colon to join the urethra at a point cephalad to the testes; in the female, the ovaries lay just caudad to the kidneys and the uterus traveled caudally, ending at the vagina.

As intra-uterine mortality is negligible from the 17th until the 22nd day (Davis and Hall, 1950) it is reasonable to assume that the sex ratio thus determined closely approximates the ratio at parturition.

The sex ratio after weaning was determined from records of over 2000 wild rats that were trapped in the residential districts of Baltimore, about 1000 in the poultry warehouses, and about 2000 rats from a farm adjacent to the city. Trapping success was not considered differential with respect to the sex of the trapped animal, since Emlen (unpublished MS) has shown that in trapping an area to extinction, the sex ratio of the trapped animals remains fairly constant throughout the period of operation.

The division of the rats into age groups presented a difficult problem because of the impossibility of determining the chronological age of a wild rat. Therefore the chronological age was discarded entirely and a new age classification was developed, based on physiological state as determined by either the state of sexual activity or the weight. Since both the weight and the length of the animal mirror to a fair extent the physiological state, and since they are among the most readily obtainable measurements that can be taken on rats, either one could be used to show the physiological age, and consequently the sexual age, of the animal. However, it must be emphasized that because rats from different localities attain sexual maturity at different weights and lengths (Davis, 1949), an age table based on either of these measurements must be specific for that particular locale alone, and comparisons between different locales must be made through the medium of com-

parative physiological states, such as birth or puberty. For example, a female rat of 88 gm. from the farm is the same physiological age as a female rat of 105 gm. from the city, since both develop perforate vaginae at that size, even though the rats were born possibly a month or more apart.

TABLE III

Sex Ratio of Wild Brown Rats with Respect to Weight Groupings

Gms.	No. of rats	Per cent. male	S.D.[1]	P[2]
		City Rats		
0-49	158	53.16	3.98	0.4
50-99	495	50.71	2.25	0.8
100-149	312	44.55	2.83	0.06
150-199	259	42.86	3.11	0.02
200-249	276	43.48	3.01	0.03
250-299	279	39.07	2.66	< 0.005
300-349	290	44.48	2.94	0.06
350-399	310	42.90	2.84	0.01
400-449	312	41.67	2.83	0.003
450-499	235	50.21	3.27	0.9
500-549	120	56.67	4.57	0.1
550-599	53	66.04	6.87	0.02
600-649	10	50.00	...	...
		Farm Rats		
0-49	203	53.20	3.51	0.4
50-99	351	39.60	2.67	< 0.005
100-149	280	44.64	2.99	0.07
150-199	288	37.50	2.95	< 0.005
200-249	282	36.88	2.98	< 0.005
250-299	316	43.35	2.81	0.02
300-349	260	56.54	3.06	0.03
350-399	130	73.08	4.39	< 0.005
400-449	42	90.48	7.72	< 0.005
450-499	1	...	...	...

[1] Standard deviation.

[2] Probability that the observed difference could be obtained by chance.

For the purposes of this paper, weight was chosen as the age-group factor, and in Table III the 5262 available rats were divided into 50-gm. weight (age) groups; sex ratios were determined for each group. (For the remainder of

this paper, the word "age" will be used in the above-defined physiological sense and will not refer to length of time, unless otherwise stated.)

In order to determine whether the general impression of a sex-ratio fluctuation gained from the grouping (based on weight) in Table III was essentially valid, the rats were regrouped according to their physiological state with no regard paid to their weights (Table IV). The first group in Table IV, "parturition," consists of embryos from 17 days of (embryonic) age until parturition; the second group, "immature," consists of male rats with abdominal testes and females with imperforate vaginae; and the last group, "mature," consists of males with scrotal testes and females with perforate vaginae.

TABLE IV

Sex Ratio of Wild Brown Rats with Respect to Sexual Maturity

Stage	No. of rats	Per cent. male	S.D.[1]	P[2]
Parturition	1,044	51.25	1.550	0.4
Immature..	1,325	56.68	1.374	< 0.005
Mature....	3,937	42.74	0.797	< 0.005

[1] Standard deviation.

[2] Probability that the observed difference could be obtained by chance.

Results and Discussion

The 118 usable tracts mentioned above contained a total of 1047 embryos, three of which were ruined by improper preservation; of the remaining 1044 embryos, 535, or 51.25 per cent. were male (Table IV). This figure is not significantly different from 50 per cent. The true sex ratio at birth can be assumed (19 chances out of 20) to lie within the range of two standard errors (46.90–53.10 per cent.). It is interesting to note that these figures closely parallel those of the albino rats shown in Table I, where King's figure of 51.26 per cent. is also not significantly different from 50 per cent.

The situation with the older rats was quite different. The sex ratios of the various weight classes are shown in Table III, but not enough 0–49-gm. rats were available from either the city or the farm to determine whether the slight increase in density of males in this group was statistically significant. However, the table does show clearly the decline in the percentage of the population that is male occurring after young adulthood (150 gm.). The probability that these sex ratios do not differ from 50 per cent. is in most cases less than 0.05.

It is necessary to consider the possibility that the low percentage of males is caused by the fact that there is a weight differential between the sexes at comparable ages. With few exceptions, the assumption can be made that the weight of a male rat is either equal to or greater than, but not less than, the weight of a non-pregnant female of corresponding age (chronological or physiological). On the basis of this assumption it follows that at any given weight we would be comparing males of one age to females of the same age or older, and since the size of a population segment decreases with the age of the segment, the sex ratio would inevitably favor males. Therefore, the percentage of male rats would actually be somewhat lower than is represented for each group in Table III. Also, in view of this argument, the higher percentage of males in the heavier weight classes (Table III) does not justify the conclusion that males live longer (chronologically) than females; in fact, the converse has proven to be the case (Davis, 1948).

The fluctuation evident in Table III is brought out more clearly in Table IV, where the confusing issue of weight is ignored. Of the immature rats, 56.68 per cent. were males and of the mature rats, 42.75 per cent. were males; both percentages are significantly different from 50 per cent. ($P < 0.005$). The percentages of males at parturition, immaturity, and maturity are significantly different from each other.

On the basis of the above data and calculations, the

hypothesis is presented that there exists in rat populations a mortality differential with respect to sex; that the mortality is greater in females before breeding age, and greater in males afterward.

Summary

The object of this paper is to determine the sex ratio of the various age segments of a population of wild brown rats, *Rattus norvegicus,* in their natural habitat.

The sex ratio at parturition was determined by the examination of 1044 embryos that were found in the reproductive tracts of 118 wild female brown rats. The age of these embryos ranged from 17 to 22 days, and since intrauterine mortality and resorption is negligible from the 17th day until parturition, the sex ratio of the embryos as determined was assumed to be comparable to the sex ratio at parturition. Of the 1044 embryos, 51.25 per cent. were male, but this figure is not significantly different from 50 per cent. ($P = 0.4$).

The sex ratio at later ages was determined from the records of over 5000 wild rats that were trapped throughout the residential slum and poultry warehouse areas of the city of Baltimore, and from a farm adjacent to the city. Since the chronological age of the trapped rats was impossible to determine, a new age classification was used, based on physiological state as determined either by the degree of sexual activity or the weight of the animal.

The 5262 rats available were grouped into 50-gram weight classes and also were divided according to the degree of sexual activity. A preponderance of females in the middle weight groups was evident ($P \leq 0.05$); this preponderance of females was not due to a weight differential between the sexes at comparable ages.

It is concluded that the proportions of the two sexes are about equal at birth (51.25 per cent. male); that the proportion of males is greater until the beginning of the reproductive age (56.68 per cent. male, $P < 0.005$); and that

throughout the adult period the proportion of females is considerably greater than that of males in the population (42.75 per cent. male, $P < 0.005$).

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ARE MOSAIC GENES SEMI-ALLELIC?

In the last issue of The American Naturalist Komai (1950) defines semi-allelic genes as "genes of similar phenotypic effect located in close sequence in the chromosome without any other intercalated gene, and crossing over, if any, very rarely occurs between them. So-called mosaic inheritance is seen, when both genes are dominant."

With this concept of semi-allelic genes, the well-known case of mammalian mosaic color pattern conforms only in part.

Black and red (or yellow) coat colors are regular Mendelian alternatives in mammals quite generally. Examples are seen in rabbits, guinea-pigs, dogs and cattle. In general black is dominant over red, but in some species a dominant red also occurs. In some species, notably rabbits and guinea-pigs, a mosaic of black and red forms a third allele, alternative both to black and to red. In the rabbit, Castle has listed four alleles at the same genetic locus, E, extended black pigmentation, E^d, dominant black (of Punnett), e, restricted black, *i.e.*, red, and e^j, Japanese. The last is shown by experiments of both Castle and Punnett to involve a mosaic condition of E^d and e, acting as a simple allele in inheritance to E, E^d, and e.

The case is similar in guinea-pigs, as analyzed by Ibsen, except that dominant black is lacking from the series, which accordingly becomes E, e, and e^p, the latter being a mosaic of E and e behaving as a unit in inheritance allelic both to E and to e. Since E is dominant to e, it is not surprising that e^p containing elements of both E and e, should be recessive to the former and dominant to the latter, as is true.

The suggestion which I offered many years ago for such situations was that a permanent union had been effected between genes normally behaving as alleles, and so incapable of occupying the same locus, such union being so intimate that it was incapable of resolution into its component

elements, yet was capable of behaving as a simple allele to either of its constituents separately.

Such a union might be interpreted as being of the nature of a "repeat," the repetition involving contrasted alleles instead of duplication of a single allele. Yet its apparent compression into the extent of a single genetic locus seems to imply a reorganization of the gene molecule (or whatever it is that constitutes a gene) so as to include both alternative determinants side by side yet inseparable.

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